M

Volume 2 Number 1 February 1956

CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

PAUL B. HOEBER, INC., PUBLISHERS, MEDICAL BOOK DEPARTMENT OF HARPER & BROTHERS

CLINICAL CHEMISTRY

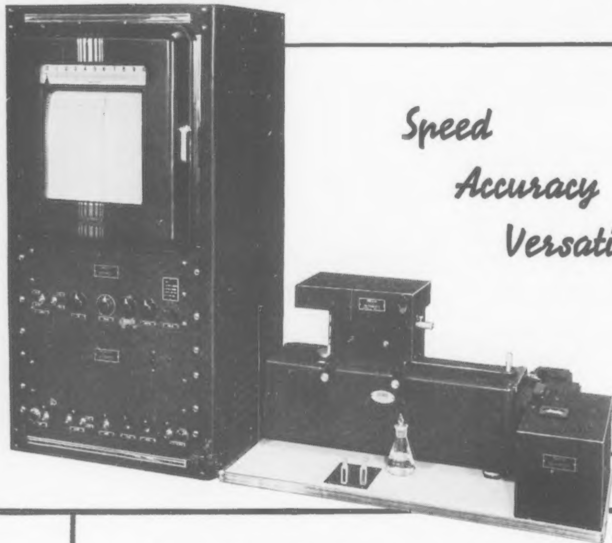
Journal of the American Association of Clinical Chemists

Scope: CLINICAL CHEMISTRY is devoted to the publication of original and review articles on the application of the science of chemistry to the better understanding of the functioning of the human organism in health and disease.

P&I *Automatic*

RECORDING SPECTROPHOTOMETER

Model RS-3*



Speed

Accuracy

Versatility

The P&I Recording Spectrophotometer RS-3 converts your present manual Beckman DU to a more sensitive line operated automatic recording instrument at a low cost.

High accuracy–stability–resolution simplify and accelerate compound identification and permit quantitative studies of analytical procedures, rapid kinetics, extinction coefficients, and differential colorimetry.

Only the P&I RS-3 provides **ALL** these unique features:

- **Linear Wavelength Scale—** no special charts or tapes.
- **Stable Zero Line**
- **Both O.D. & % Abs. are linear** 4 stand. & high sens. scales
- **Constant resolution**
- **Uninterrupted scan, 210-750 mμ.** no moving light sources
- **Automatic marking— every 20 mμ.**
- **Low-Med-High Scan Speeds**
- **Time Scale for Kinetics**
- **Line Operated**

p&i

For details, write:

PROCESS & INSTRUMENTS

15 Stone Ave., Brooklyn 33, N. Y.

*Patent Pend.

CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

Information for Authors

Original manuscripts will be considered for publication with the understanding that they are contributed solely to CLINICAL CHEMISTRY. Address manuscripts to the Chairman of the Editorial Board, Harold D. Appleton, CLINICAL CHEMISTRY, Box 123, Lenox Hill Station, New York 21, New York.

The original (on bond paper) and first carbon of the manuscript should be submitted, triple spaced with ample margins all around. The author's last name should appear on each page. Separate pages should be used for title page (with author's name and affiliations), references, footnotes (when unavoidable), illustration legends, tables, and other inserts.

References and the design of tabular matter should follow exactly the form used in current issues of this journal. Journal abbreviations should conform to the style of *Chemical Abstracts*. The accuracy of the references and the provision of adequate bibliographic data are the responsibility of the author.

A reasonable allowance is made for black and white line cuts and tabular composition. If this allowance is exceeded, or if halftones or color plates are required, special arrangements will have to be made with the Chairman of the Editorial Board.

Reprints *must* be ordered when returning proof. No free reprints are furnished.

General Information

Communications: All subscription and advertising inquiries should be addressed to the publishers: PAUL B. HOEBER, INC., Medical Book Department of Harper & Brothers, 49 East 33rd Street, New York 16, New York.

Subscription Rates: CLINICAL CHEMISTRY is issued bimonthly, one volume per year. The subscription price, payable in advance, is $8.00 in the U. S. A., possessions, and countries in the Pan-American Postal Union, $8.50 per year in Canada, $9.00 per year elsewhere. All subscriptions commence with the first issue of the current volume. Single copies $1.50. Back issues will be supplied at the single copy rate when available.

CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

Scope: CLINICAL CHEMISTRY is devoted to the publication of original and review articles on the application of the science of chemistry to the better understanding of the functioning of the human organism in health and disease.

Index to Advertisers

CLINICAL CHEMISTRY

Journal of the American Association of Clinical Chemists

VOLUME 2 **FEBRUARY 1956** **NUMBER 1**

CONTENTS

INTERNATIONAL CONGRESS ON CLINICAL CHEMISTRY

Hotel New Yorker, New York, September 9–14, 1956

Protein Metabolism During Healing of Wounds

Martin B. Williamson

Although the problem of the healing of wounds has commanded much interest for a long time, the metabolic changes which accompany wound healing have been seriously investigated only since the early work of Howes and his associates (1–4) has appeared. Much of the literature in this field has been concerned with an empirical approach to the acceleration of the healing processes. The lack of quantitatively precise methods for measuring the rates of healing, even in experimental wounds, has impaired the value of much of this work. Nonetheless, a number of metabolic implications can be gleaned from these empirical studies. Only relatively recently have systematic studies been undertaken on metabolism after injury.

The biochemical work on wounds and wound healing has been devoted largely to the metabolism of proteins by the injured organism and the determination of the character and metabolism of certain proteins and polysaccharides of the wound area. The latter have been of interest because of their close association with the protein fibers found not only in wound tissue but also in connective tissue in general. The present discussion will be limited to the consideration of some aspects of the metabolism of proteins, and their constituent amino acids, which are influenced by the infliction of various types of trauma.

NITROGENOUS CONSTITUENTS OF THE BLOOD

Disturbances in protein metabolism are usually reflected in changes of the level of the various nitrogen-containing compounds found in the different fractions of the blood. Since protein metabolism is known to

---

From the Department of Biochemistry, School of Medicine and Graduate School, Loyola University, Chicago, Ill.

The author is pleased to acknowledge the support of the U. S. Navy, Office of Naval Research (Nonr-06300), and the U. S. Public Health Service (RG-4440) for the studies carried out in his laboratory which are discussed in this paper.

be profoundly affected after trauma, it is not unexpected that changes in the level of the plasma constituents have been observed during the healing of wounds.

Plasma Proteins

The studies on the total plasma proteins have not always yielded consistent results (5, 6). These inconsistencies are probably attributable to two principal causes: (1) the differences in severity of injury considered in different reports and (2) the possibility that the concentration of some fractions of the plasma proteins might be increasing while others are decreasing. The latter has been definitely implicated as one reason for the inconsistencies observed, although the former has not been ruled out entirely. By the use of electrophoretic technics, the changes in the levels of the plasma protein fractions have been clearly and repeatedly demonstrated.

Albumin

Data from a number of laboratories have indicated that there is a small drop in the level of the plasma albumin directly after injury (7–12, 109). The decrease in the plasma albumin concentration appears to be in the neighborhood of 10–20 per cent of the pretraumatic level. All of these experiments were carried out on animals in which the quantity of plasma proteins prior to wounding might be considered to be in the normal range. Since it has been shown that in the presence of a low plasma protein level the rate of healing is much inhibited (19, 38), it would be of interest to know whether injury would result in a further decrease in the albumin level when hypoproteinemia is present before the trauma is made.

Globulin and Lymph Proteins

Accompanying the decrease in plasma albumin is a concomitant rise in the total protein content of the lymph. This increase is due not only to an increase in the amount of albumin, but also of the globulins as well (7, 10). Evidently the permeability characteristics in the injured area are altered so that the plasma proteins may pass into the lymph more easily. This effect is particularly evident when the trauma is a burn. The proteins in the lymph, of course, are more readily available for utilization in the regenerative and reparative processes connected with the healing of the trauma. Perlmann *et al.* (7) indicated that after injury a new protein appears in the lymph with an electrophoretic mobility even lower than that of the γ-globulins at pH levels above 7.0.

It would seem quite probable that this new protein in the lymph arises from the injured tissues. In these studies (7, 8, 9, 12) there was also noted a sharp increase in the α-globulins after injury, while the levels of the β- and the γ-globulins remained essentially unchanged from the normal.[1]

A most careful and extensive study on the changes in the plasma protein levels after various injuries was carried out by Chanutin and his co-workers (13–16). They fractionated the plasma from normal rats and from those which were injured in various ways, using the alcohol–low temperature technic described by Cohn *et al.* (17), before determining the amounts of the different plasma proteins in their fractions by electrophoresis. The results they obtained were quite similar to those previously noted—that is, a decrease in albumin, a marked increase in the α-globulins (and particularly in the α_1-globulins), and no significant change in the other globulin fractions. The concentration of the plasma proteins seemed to return to approximately normal values in about 10–15 days after the injuries were made. Among other items, they noted that after fractionation the plasma from the injured rats showed new spikes in the electrophoretogram of the α- and the β-globulins. These results may be interpreted to mean that either that new proteins appear in the plasma after injury or that some component of the α- and the β-globulin complexes increases markedly after injury.

Lipoproteins

The presence of most, if not all, of the serum lipoproteins in the α-globulin and β-globulin fractions has stimulated the study of these components of the plasma of injured animals (12, 18). These reports indicate that after injury there is a small rise, which may not be significant, in the plasma lipoproteins having a low S_f, while those having a high S_f increase about two or three times over that found in the plasma of normal animals. The implications of these changes in the lipoprotein levels is obscure.

Fibrinogen

The fibrinogen content of the plasma has been shown to increase very markedly after injuries (8, 20, 21). This increase reaches a peak of about 70 per cent above normal levels in 1–2 days after injury and then gradually returns to normal over the course of several weeks. Although the

[1] It has been pointed out that these changes in plasma protein levels appear to be relatively nonspecific since they appear in all situations where there is inflammation or destruction of tissue, regardless of cause (8).

clotting time of the blood appears to remain normal, the blood prothrombin level has been reported to be somewhat lower than normal shortly after injury (21).

Hemoglobin

It has also been shown that there is a decrease in the hemoglobin content of the blood after burns (8, 22, 23, 24). It is well established that a low or decreasing hemoglobin level usually acts as a stimulus for the production of more hemoglobin. In spite of the expected stimulation of the low hemoglobin levels, studies on the synthesis of heme with N^{15}-labeled glycine in injured animals indicated that there was a marked depression in the formation of hemoglobin for as long as 25 days after injury. The excretion of increased amounts of urobilinogen and coproporphyrin by the experimental animals during these studies (24) suggests the possibility of an increased destruction of hemoglobin as well.

Blood Enzymes

The results of investigations on the level of several enzymes in the blood after injuries have also been reported. It has been shown that the plasma lipase level decreases after burns, but returns to normal levels upon recovery (25). After severe injuries the carbonic anhydrase of the blood was reported to be much decreased. Slighter injuries caused no detectable depression in the level of the blood carbonic anhydrase (26). Zamencik *et al.* (27) have demonstrated the presence of a peptidase, capable of hydrolyzing several synthetic peptides, in the lymph and serum of dogs. This peptidase activity increases in both fluids to a significant extent after injury. The possibility exists that this increase in proteolytic enzyme might result in an increased amount of protein fragments in the blood. However, it has been reported that experimental burns do not give rise to polypeptidemia, as measured by the difference between the substances precipitable by tungstic acid and by trichloroacetic acid (28). Other reports have indicated that there is some rise in the peptide level of the blood after severe injury (29, 30).

Amino Acids

Man *et al.* (31) have reported finding a sharp depression in the plasma amino acid level after surgical trauma. This decreased amino acid level was prolonged until healing was largely completed. Data from other reports (32, 33) have indicated that there is a small decrease in the level of some amino acids, and especially some of the "essential" ones, in the plasma after wounding. In these studies (32, 33) the differences that were indi-

cated seemed to be more related to the nutritional state than to the trauma. Several papers indicate that there is an increase in the plasma amino acid levels in several species after burns (9, 34–37). Although the level of several amino acids increased in the plasma after burns, there were particularly large increases in the histidine, tyrosine, and phenylalanine levels. However, the largest part of the increased plasma amino acid fraction was due to the presence of a compound which behaves, chromatographically, like taurine, a metabolite of cystine[2] (36). Increases in the plasma urea (36, 109) and uric acid (12, 109) have also been observed following trauma.

NITROGEN METABOLISM

The most striking change of protein metabolism which results from injuries is the appearance of a negative nitrogen balance, frequently even in young or growing animals. Practically every paper considering metabolic aspects of the healing of wounds has demonstrated or noted the posttraumatic negative nitrogen balance. The increase in excreted nitrogen seems to be due primarily to an increase in the excretion of urea, although somewhat larger than normal amounts of amino acids, creatine, ammonium ion, and uric acid may also appear in the urine (9, 39–43). It has also been reported that there is a large increase in the "undetermined" nitrogen of the urine after burns (37, 42).

A chromatographic analysis has been made of the amino acids excreted in the urine after burns and surgical trauma (41). The rise in the amounts of amino acids excreted appears to be roughly correlated with the severity of the trauma. Although some increased excretion of all the amino acids was noted, the greatest increase appeared to be in the high-molecular-weight "essential" amino acids. Care must be taken in interpreting many of the results reported in papers such as that noted above, since the pattern of urine constituents may be markedly altered by unrelated stimuli. For example, barbiturates will cause a decrease in urea excretion with a concomitant increase in amino acid excretion in both normal and wounded animals (48).

Protein Metabolism

The metabolism of proteins gives rise to excretory products containing sulfur, as well as nitrogenous ones. The excretion of these sulfur-contain-

[2] In this discussion, the use of the word "cystine" implies both cystine and cysteine, unless otherwise specifically noted. This usage may be considered permissible at present, since the reduced and oxidized forms of this amino acid are interconvertible *in vivo*, and since the functional form of the amino acid in the processes under discussion is still undetermined.

ing compounds might be expected, and have been shown, to increase during the negative nitrogen balance observed after experimental wounds have been inflicted (43–46, 50). However, the amount of sulfur excreted was noted to be less than the nitrogen/sulfur ratio in the body tissues would lead one to expect, indicating a net retention of sulfur.

Effect of Protein on Wound Healing

Many experiments have been reported in which attempts have been made, by various methods, to decrease the negative nitrogen balance and also to increase the rate of healing of the trauma studied. The expected protein-sparing effect of a high-carbohydrate diet does not seem to be effective in decreasing the excretion of nitrogen or altering the rate of healing of wounds. However, the feeding of a high-protein diet not only increased the rate of healing of wounds over that displayed when a low protein diet was fed, but also resulted in a closer approach to nitrogen balance (2, 44–46, 54–63, 71, 74). Results which might be considered typical of this kind of experiment are shown in Fig. 1. It is most important to realize that the healing of wounds will take place when a very low protein diet is fed, and even in fasting animals (3, 44, 47, 60–63, 70, 71). This is also illustrated in the data presented in Fig. 1, where there is a large increase in tensile strength (the measure of healing) of the wounds even in the animals receiving the low-protein diet. In order

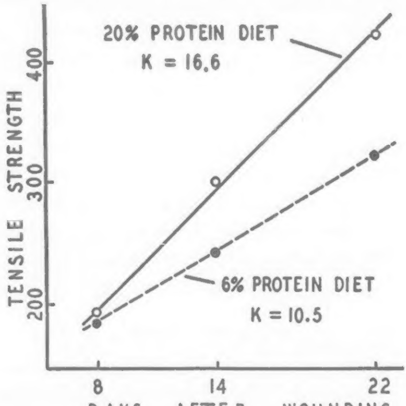


Fig. 1. The rate of healing of experimental wounds in rats fed isocaloric amounts of a high- and low-casein diet. The K values represent the slopes of the lines and are a numerical evaluation of the rate of healing. Data from Williamson, McCarthy, and Fromm (44).

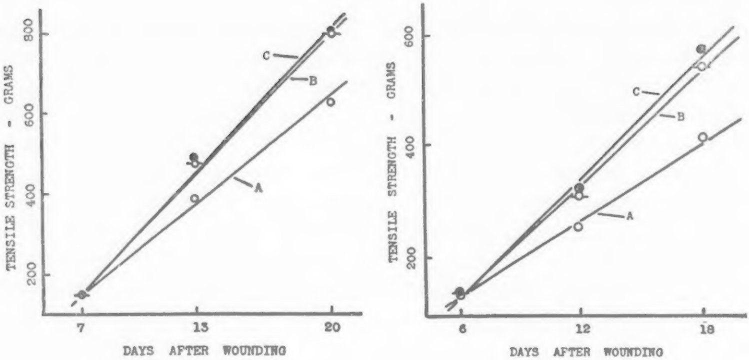
to inhibit wound healing completely an appreciable period of starvation seems necessary so that a large part of the body weight is lost prior to the infliction of the wound (65). With this information in mind, one is tempted to suggest that the materials used for the regeneration of wound-tissue protein comes primarily, if not exclusively, from the tissue protein.

The mere feeding of a high-protein diet, without taking into account the constituents of the protein, is not sufficient to promote an increased rate of wound healing. Thus, feeding wounded animals with a diet which was high in protein (gelatin) did not significantly alter the rate of healing or the nitrogen balance from that observed when a low-casein diet was fed (44, 45). It then seems obvious that it is not the excess protein *per se* which is involved in increasing the rate of wound healing, but rather that the extra protein in the high-protein diet must be providing a larger amount of one or more amino acids which are in short supply, or which are required to a larger extent for the healing processes. As a corollary, it is reasonable to suppose that the requirements of the healing processes for relatively excessive amounts of one or more amino acids, supplied by the tissue proteins, leaves large quantities of the remaining amino acids in such a balance that they cannot be used for protein synthesis (68), but can only be metabolized for energy purposes. The residual nonutilizable amino acid nitrogen is then excreted and results in the negative nitrogen balance found after injury.

Effect of "Essential" Amino Acids

The effect of several "essential" amino acids on the healing of wounds and the excretion of nitrogen during this time has been studied. Lysine, tryptophane, and valine were found to have no significant effect on the rate of healing of experimental wounds when used to supplement a low- or high-protein diet (55). Similarly, a histidine supplement to a low-protein diet has no effect on the rate of healing or on the negative nitrogen balance (52). The administration of methionine to wounded or burned animals has been shown to accelerate the rate of healing and to decrease the excretion of nitrogen (6, 46, 49, 50, 66, 67, 73). The administration of cystine, *but not cysteine*, produces the same effect as that shown by methionine (47, 66, 67) (Fig. 2). The mechanism which makes cystine utilizable, but cysteine of no value, for the healing processes is not known.

The over-all reaction for the conversion of methionine to cystine in vivo has been demonstrated to be irreversible (75–77). It then follows



A *B*

Fig. 2*A*. The rate of healing of experimental wounds in rats fed a 6% casein diet supplemented with equimolar amounts of different amino acids. (*A*) Alanine supplemented to the diet; (*B*) methionine supplement; (*C*) cystine supplement. Data from Williamson and Fromm (47). **2*B*.** The same as the above except that the basal diet contained no protein (47).

that cystine is the principal amino acid in short supply during the period of wound healing; the negative nitrogen balance may be considered to be the result of an attempt to meet the requirement for an extra supply of cystine. The ability of methionine to increase the rate of healing and to decrease nitrogen excretion in wounded animals would seem to be due to the fact that it can be readily converted to cystine by the injured organism. Of course, this does not infer that the other amino acids are not required for the processes involved in the healing of wounds.[3]

The retention of sulfur by the wounded animal, the excess requirement for cystine, and its ability to increase the rate of healing all point to the possibility that the cystine is utilized primarily in the synthesis of protein. Since the injured animal displays a marked protein catabolism and the principal anabolic activity occurs in the wound area, it would be expected that the cystine was being used for the production of wound proteins. A further discussion of the proteins in regenerating wound tissue will be considered in the section "Wound Proteins."

---

[3] It has recently been reported that cystine is required for the extended growth of fibroblasts in vitro (110). Fibroblasts may survive for several days without any of the sulfur amino acids being present in the growth medium. However, the presence of cystine alone, but not methionine alone, was shown to extend the growth period to more than a month. The best growth was observed when both amino acids were present. These results are a a further confirmation of the importance of cystine during the regeneration of tissue and growth.

Metabolic Reactions

The general metabolic reactions of injured rats have been studied with the use of N^{15}-labeled glycine (78) and C^{14}-labeled glycine (79). In both of these series of experiments, it was shown that the labeled compounds never reached as high a level in the visceral organs of the injured animals as in the normal unwounded controls. This effect may be due either to a reduced uptake or to a more rapid turnover of the labeled atom. The latter situation would seem to be indicated from the fact that the injured animals respired more $C^{14}O_2$ than did the controls. Experiments using methionine and cystine labeled with S^{35} indicate that the rate of sulfur metabolism is increased during the healing of wounds (49–51), the turnover of the S^{35} being more rapid in injured animals as compared with the normal controls. Some of the results of these experiments seem to suggest that methionine from the liver supplies a large part of the cystine required by the regenerating wound tissue.

The metabolic activity in the liver after injuries has also received some attention. It has been shown that the deamination of alanine at the height of nitrogen excretion after burns is quite appreciably lower than in control animals (78). This may account in part for the increased excretion of amino acids observed after wounding (41, 109). It has also been reported that the liver lipids drop markedly from normal levels for several days after injury (81). This reduction in liver lipids is reported to be, at least partially, reversed by the administration of methionine or cystine. It might be postulated that the decrease in liver lipids and the increased level of plasma lipoproteins (12, 18) are merely two aspects of the same phenomenon. In spite of the reported changes in the liver constituents and metabolism, it has been shown that the oxygen consumption of liver slices from burned animals is much the same as that found in normal animals (82).

Since several hormones are known to affect the metabolism of proteins, a short discussion of the effect of some of these hormones on wound healing would be in order. It has been demonstrated that when thyroidectomized animals are wounded they excrete considerably less nitrogen than do wounded nonthyroidectomized controls, while no appreciable change in the rate of healing is observed. On the other hand, the administration of thyroxin or pituitary thyrotrophic hormone depresses the rate of healing markedly, but only slightly increases the excretion of nitrogen (83–89). Although some of the androgenic steroids appear to depress the excretion of nitrogen to some extent after wounding, they also inhibit the rate of healing (84, 85, 89–92). Pituitary growth hormone appears to

inhibit the rate of healing when administered at relatively high dosage levels, but increases the healing rate when smaller amounts are given (53, 89). Finally, several of the steroids from the adrenal cortex have been shown to have a strongly inhibitory effect on the healing processes, but appear to have little, if any, effect on the posttraumatic excretion of nitrogen (93–99).

WOUND PROTEINS

The work on the structures and the transformations which occur in wound tissue has been approached, for the most part, from the histologic point of view. Still, there has been some information accumulated about the chemical characteristics of the substances being synthesized in the regenerating wound tissue. An extensive study has been reported by Orekhovitch (100) on two of the important proteins which appear in regenerating wound tissue. One of the early proteins to appear in the wound tissue is a procollagen "which has an intrinsic role in the regeneration processes." The procollagen serves as a precursor and substrate for the formation of collagen fibers, found in the healing wound. Both the procollagen and collagen appear to have similar properties to collagens and procollagens isolated from sources other than regenerating wound tissue. An amino acid analysis of the purified proteins was also reported.

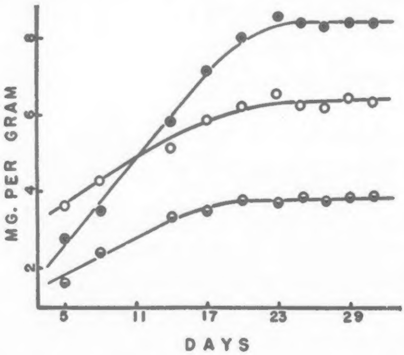


Fig. 3. The concentration of nitrogen, methionine, and cysteine and cystine in the regenerating wound tissue of rats plotted against days after wounding. Methionine values are represented by open circles; nitrogen by half-open circles; cysteine and cystine by solid circles. All the values are shown in terms of milligrams per Gram of wet tissue, except for the nitrogen, which is in terms of 0.1 of actual value. Data from Williamson and Fromm (51).

The wound collagen and procollagen consist largely of the prolines and the simpler amino acids; they do not contain all of the common amino acids; they contain only a small percentage of methionine and little, if any, cystine.

Cystine-Methionine Content of Wound Tissue

Some of the proteins which are synthesized in the wound tissue appear to contain appreciably more cystine and methionine than does either the normal skin tissue, the wound collagen, or procollagen. The regenerating wound tissue, at the maximum level, has been shown to contain 170 mg. of methionine and 220 mg. of cystine per Gm. of nitrogen as compared with 115 mg. of methionine and 75 mg. of cystine per Gm. of nitrogen in normal skin (51). From the rate of deposition of the sulfur-containing amino acids and nitrogen, shown in Fig. 3, it can be seen that there are two distinct types of sulfur-rich proteins being produced in the regenerating wound tissue. During the early stages of healing, the wound tissue is accumulating a larger proportion of proteins that are relatively high in methionine and low in cystine. As the healing progresses, proteins having a higher percentage of cystine than methionine are synthesized and deposited in the healing wound tissue.

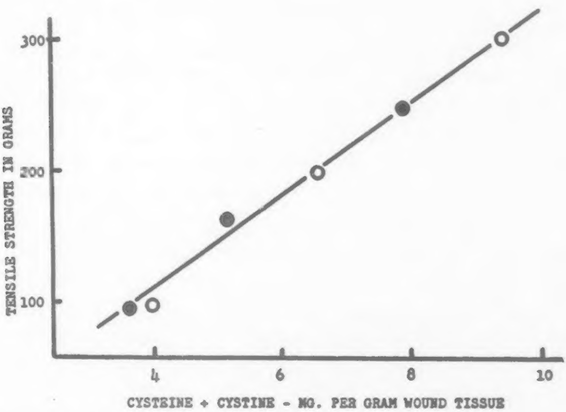


Fig. 4. Tensile strength of healing wounds in rats fed a protein-free diet plotted against the cystine and cysteine content of the regenerating wound tissue. Open circles are data from rats given a methionine supplement in the diet. Solid circles represent data from rats given an equimolar supplement of alanine in the diet. Data from Williamson and Fromm (46).

Since the strength of wound tissue appears to be due to its content of collagen fibers, the methods for measuring the rate of healing of wounds which employ a tensile-strength or wound-rupture technic are actually measuring the rate of deposition of collagen fibers in the wound area. In spite of the lack of cystine in collagen, there appears to be an excellent correlation between the amount of cystine deposited in the proteins of the wound tissue and the tensile strength of the wound, as shown in Fig. 4. This may be explained by assuming that the cystine-rich proteins are being produced in the wound tissue at approximately the same rate as is the collagen.

Sulfur and Sulfate Uptakes

The uptake of sulfur in the form of amino acids must not be confused with the uptake of sulfate ion by the healing wound, which has also been reported (101, 102, 111). While the sulfur amino acids are used for the synthesis of protein, the sulfate ion is incorporated into the mucopolysaccharides of the ground substance associated with the collagen fibers of the wound tissue (103). However, the sulfate ion produced from the metabolism of the sulfur-containing amino acids may be used for the latter function.

Enzymatic Proteins

Proteins with enzymatic properties have also been identified in the regenerating wound tissue. A proteolytic enzyme has been shown to be present in wound tissue by many workers (40, 104, 105). Whether this proteolytic enzyme is the same as the one detected in the plasma (27), and whether it originates in the body tissues and migrates to the wound tissue, or vice versa, is not known. At present, we can only guess at the function of this enzyme in the economy of the wound tissue. The healing wound tissue has also been shown to have a high level of phosphatase activity (106, 107). Since collagen fibers have been shown to bind phosphatases very strongly in vitro (108), it is possible that the origin of this enzyme in the wound tissue is due to its accumulation from body tissue sources. Again, in this case, the origin and function of this enzyme can only be surmised.

CONCLUSIONS

The foregoing is illustrative of the available information on the alterations in the biochemical processes which occur during the healing of wounds. Many of the studies mentioned appear to make some aspects

of the phenomena involved in the healing of wounds more understandable. Yet, these results are too isolated to form a clear and unified picture of the healing processes and the mechanisms which control them. It will be necessary to discover the mechanisms which bring about the changes in metabolism before a real understanding of the processes of wound healing can be obtained. Obviously, much more data are required before these mechanisms can become apparent.

REFERENCES

1. Howes, E. L., and Harvey, S. C., *J. Exp. Med.* **53,** 577 (1932).
2. Harvey, S. C., and Howes, E. L., *Ann. Surg.* **91,** 641 (1930).
3. Howes, E. L., Briggs, H., Shea, R., and Haevey, S. C., *Arch. Surg.* **27,** 846 (1933).
4. Howes, E. L., Sooy, J. W., and Harvey, S. C., *J. Am. Med. Assoc.* **92,** 42 (1929).
5. Arey, L. B., *Physiol. Rev.* **16,** 327 (1936).
6. Localio, S. A., Chassin, L., and Hinton, J. W., *Surg. Gynecol. Obstet.* **86,** 106 (1948).
7. Perlmann, G. E., Glenn, W. W. L., and Kaufman, D., *J. Clin. Invest.* **22,** 627 (1943).
8. Shedlovsky, T., and Scudder, J., *J. Exp. Med.* **75,** 119 (1942).
9. Glenn, W. W. L., Muus, J., and Drinker, C. K., *J. Clin. Invest.* **22,** 451 (1943).
10. Cope, O., *et al.*, *Ann Surg.* **128,** 1041 (1948).
11. Tanamura, H., *Japan. J. Med. Sci.* **5,** 91 (1944).
12. Schilling, J. A., *et al.*, *Proc. Soc. Exp. Biol. Med.* **89,** 189 (1955).
13. Chanutin, A., and Gjessing, E. C., *J. Biol. Chem.* **165,** 421 (1946).
14. Gjessing, E. C., and Chanutin, A., *J. Biol. Chem.* **165,** 413 (1946).
15. Gjessing, E. C., Ludewig, S., and Chanutin, A., *J. Biol. Chem.* **170,** 551 (1947).
16. Gjessing, E. C., Chanutin, A., and Floyd, C. S., *J. Biol. Chem.* **169,** 657 (1947).
17. Cohn, E. J., *et al.*, *J. Am. Chem. Soc.* **68,** 459 (1946).
18. Thomas, R. S., Vaughn, B. E., Walker, E. L., and Pace, N., *Proc. Soc. Exp. Biol. Med.* **85,** 553 (1954).
19. Thompson, W. D., Ravdin, I. S., and Fran, I. L., *Arch. Surg.* **36,** 500 (1938).
20. Kapp, R. W., and Shaw, D. T., *Surg. Gynecol. Obstet.* **94,** 577 (1952).
21. Campbell, D. A., Gabriel, L. T., and Van Hoek, D. E., *Surg. Forum, 36th Clin. Cong. Am. Coll. Surg.* 515 (1951).
22. Brathwaite, F., and Moore, F. T., *Brit. J. Plastic Surg.* **1,** 81 (1950).
23. James, G. W., Purnell, O. J., and Evans, E. I., *J. Clin. Invest.* **30,** 181 (1951).
24. James, G. W., Abbott, L. D., Brooks, B. W., and Evans, E. I., *J. Clin. Invest.* **33,** 150 (1954).
25. Sabatini, C., *Arch. ital. dermatol.* **24,** 241 (1951).
26. Heiman, E. Y., *Byull. Eksptl' Biol. Med.* **22,** 56 (1946).
27. Zamencik, P. C., Stephenson, M. L., and Cope, O., *J. Biol. Chem.* **158,** 135 (1945).
28. Schummeldorfer, N., *Arch. exptl. Pathol. Pharmakol.* **207,** 82 (1949).
29. Lambret, O. J., Dreissens, J., and Warembourg, H., *Compt. rend. soc. biol.* **123,** 10 (1936).
30. Duval, P., Roux, J., and Goiffon, H., *Presse méd.* **42,** 1785, (1934).
31. Man, E. B., Bettcher, P. G., Cameron, C. M., and Peters, J. P., *J. Clin. Invest.* **25,** 701 (1946).
32. Everson, T. C., and Fritschel, M. J., *Surgery* **30,** 931 (1951).
33. Everson, T. C., and Fritschel, M. J., *Surgery* **31,** 226 (1952).
34. Harkins, H. N., and Long, C. N. H., *Am. J. Physiol.* **144,** 661 (1945).
35. Levenson, S. M., *et al.*, *New Engl. J. Med.* **235,** 467 (1946).

36. Rosen, H., and Levenson, S., *Proc. Soc. Exp. Biol. Med.* **83,** 91 (1952).
37. Walker, J., *Surgery* **19,** 825 (1946).
38. Rhoads, J. E., Fliegelman, M. T., and Panzer, L. M., *J. Am. Med. Assoc.* **118,** 21 (1942).
39. Clark, J., Peter, R. A., and Rossiter, R. J., *Quart. J. Physiol.* **33,** 113 (1945).
40. Peters, R. A., *Brit. Med. Bull.* **3,** 81 (1945).
41. Nardi, G. L., *J. Clin. Invest.* **33,** 847 (1954).
42. Taylor, F. H., Levenson, S. M., Davidson, C. S., and Adams, M. A., *New Engl. J. Med.* **229,** 855 (1943).
43. Williamson, M. B., McCarthy, T. H., and Fromm, H. J., unpublished data (1952).
44. Williamson, M. B., McCarthy, T. H., and Fromm, H. J., *Federation Proc.* **10,** 270 (1951).
45. Williamson, M. B., McCarthy, T. H., and Fromm, H. J., *Proc. Soc. Exp. Biol. Med.* **77,** 302 (1951).
46. Williamson, M. B., and Fromm, H. J., *Proc. Soc. Exp. Biol. Med.* **83,** 329 (1953).
47. Williamson, M. B., and Fromm, H. J., *Proc. Soc. Exp. Biol. Med.* **80,** 623 (1952).
48. Williamson, M. B., McCarthy, T. H., and Fromm, H. J., *Arch. Biochem. and Biophys.* **32,** 226 (1951).
49. Williamson, M. B., and Fromm, H. J., *Federation Proc.* **13,** 322 (1954).
50. Williamson, M. B., and Fromm, H. J., *Proc. Soc. Exp. Biol. Med.* **87,** 366 (1954).
51. Williamson, M. B., and Fromm, H. J., *J. Biol. Chem.* **212,** 705 (1955).
52. Williamson, M. B., and McCarthy, T. H., unpublished data (1952).
53. Williamson, M. B., and Neumann, G. J., *Federation Proc.* **13,** 323 (1954).
54. Abbott, W. E., Hirshfeld, J. W., Williams, H. H., and Pilling, M. A., *Surgery* **20,** 284 (1946).
55. Morris, H. P., Dubnik, C. S., and Dunn, T. B., *J. Nat. Cancer Inst.* **5,** 271 (1945).
56. Kobak, M. W., Benditt, E. P., Wissler, R. W., and Steffee, C. H., *Surg. Gynecol. Obstet.* **85,** 751 (1947).
57. Localio, A. S., Cassale, W., and Hinton, J. W., *Surg. Gynecol. Obstet.* **77,** 369 (1943).
58. Chalkley, H. W., Algire, G. H., and Morris, H. P., *J. Nat. Cancer Inst.* **6,** 363 (1946).
59. Sandblom, P., Petersen, P., and Muren, A., *Acta Chir. Scand.* **105,** 252 (1953).
60. Green, J. W., Hearn, G. R., and Allison, J. B., *Federation Proc.* **12,** 415 (1953).
61. Charney, J., Williamson, M. B., and Bernhard, F. W., *Science* **105,** 396 (1947).
62. Co Tui, C., *et al.*, *Ann. Surg.* **119,** 815 (1944).
63. Williams, R. W., Mason, L. B., and Bradshaw, H. H., *Surg. Forum, Proc. 36th Clin. Cong. Am. Coll. Surg.* **410** (1951).
64. Meyer, F. L., Hirschfeld, J. W., and Abbott, W. E., *J. Clin. Invest.* **26,** 796 (1947).
65. Findlay, C. W., and Howes, E. L., *New Engl. J. Med.* **246,** 597 (1952).
66. Croft, P. B., and Peters, R. A. *Nature* **155,** 175 (1945).
67. Croft, P. B., and Peters, R. A., *Lancet* **166** (1945).
68. Cannon, P. R., *et al.*, *Federation Proc.* **6,** 390 (1947).
69. Grossman, M. F., Calloway, D. H., Bowman, J., and Calhoun, W. R., *Federation Proc.* **13,** 459 (1954).
70. Varco, R. L., *Surg. Gynecol. Obstet.* **84,** 611 (1947).
71. Rhoads, J. E., *Federation Proc.* **11,** 659 (1952).
72. Localio, S. A., Morgan, M. E., and Hinton, J. W., *Surg. Gynecol. Obstet.* **86,** 582 (1948).
73. Tamayo, R. P., and Ihnen, M., *Am. J. Pathol.* **29,** 233 (1953).
74. Cuthbertson, D. P., *Proc. Nutr. Soc.* (*Engl. and Scot.*) **4,** 185 (1946).
75. du Vigneaud, V., Kilmer, G. W., Rachelle, J. R., and Cohn, M., *J. Biol. Chem.* **155,** 645 (1944).
76. Binkley, F., and Okeson, D., *J. Biol. Chem.* **182,** 273 (1950).
77. Binkley, F., *J. Biol. Chem.* **155,** 39 (1944).
78. Roth, J., *Am. J. Physiol.* **176,** 471 (1954).

79. Lee, W., and Alpen, E. L., *Federation Proc.* **13,** 249 (1954).
80. van Bekkum, D. W., and Peters, R. A., *Quart. J. Physiol.* **36,** 127 (1951).
81. Picard, J., and Cartier, P., *Compt. rend. soc. biol.* **143,** 678 (1949).
82. van Bekkum, D. W., Lathe, G. H., and Peters, R. A., *Quart. J. Physiol.* **35,** 321 (1950).
83. Sellers, E. A., You, S. S., and You, R. W., *Endocrinology* **47,** 148 (1950).
84. Grebble, M. de G., Peters, R. A., and Wakelin, R. W., *Quart. J. Physiol.* **36,** 119 (1951).
85. Williamson, M. B., and Neumann, G. J., unpublished data.
86. Wase, A. W., Eichel, H. J., and Rapplinger, E., *Proc. Soc. Exp. Biol. Med.* **84,** 152 (1953).
87. Moltke, E., *Proc. Soc. Exp. Biol. Med.* **88,** 596 (1955).
88. Smelzer, G. K., and Ozanics, V., *J. Cellular Comp. Physiol.* **43,** 107 (1954).
89. Taubenhaus, M., and Amromin, G. D., *J. Lab. Clin. Med.* **36,** 7 (1950).
90. Braasch, J. W., Wakerlin, G. E., Ball, J. H., and Levenson, S., *Proc. Soc. Exp. Biol. Med.* **75,** 264 (1950).
91. Taubenhaus, M., Taylor, B., and Morton, J. V., *Endocrinology* **51,** 183 (1952).
92. Taubenhaus, M., and Amronin, G. D., *Endocrinology* **44,** 359 (1949).
93. Chassin, J. L., *et al. Proc. Soc. Exp. Biol. Med.* **86,** 446 (1954).
94. Spain, D. M., and Molomut, N., *Proc. Soc. Exp. Biol. Med.* **83,** 346 (1953).
95. Taubenhaus, M., Jacobson, M., Morton, J. V., and Levine, R., *Proc. Soc. Exp. Biol. Med.* **84,** 646 (1953).
96. Ingle, D. J., Ward, E. O., and Kuizenga, M. H., *Am. J. Physiol.* **149,** 510 (1947).
97. Noble, R. L., and Toby, C. G., *J. Endocrinol.* **5,** 303 (1947).
98. Shapiro, R., Taylor, B., and Taubenhaus, M., *Proc. Soc. Exp. Biol. Med.* **76,** 854 (1951).
99. Ragen, C., *et al.*, *Proc. Soc. Exp. Biol. Med.* **72,** 718 (1949).
100. Orekhovitch, V. N., *Ukrain. Biokhim. Zhur.* **22,** 456 (1950).
101. Layton, L. L., *Proc. Soc. Exp. Biol. Med.*, **73,** 570 (1950).
102. Upton, A. C., Odell, T. T., and Gude, W. D., *Federation Proc.* **14,** 421 (1955).
103. Meyer, K., "The Chemistry of Ground Substances of Connective Tissue," *in* Asboe-Hansen, G., (Ed.): *Connective Tissue in Health and Disease.* Copenhagen, Denmark, Munksgaard, 1954.
104. Neville-Jones, D., and Peters, R. A., *Biochem J.* **43,** 303 (1948).
105. Ungar, G., and Damgaard, E., *Proc. Soc. Exp. Biol. Med.* **87,** 378 (1954).
106. Fisher, I., and Glick, D., *Proc. Soc. Exp. Biol. Med.* **66,** 14 (1947).
107. French, J. E., and Benditt, E. P., *Arch. Pathol.* **57,** 352 (1954).
108. Gold, N. I., and Gould, B. S., *Arch. Biochem. and Biophys.* **33,** 155 (1951).
109. Williamson, M. B., *et al.*, unpublished data (1954).
110. Morgan, J. F., and Morton, H. J., *J. Biol. Chem.* **215,** 539 (1955).
111. Kodicek, E., and Loewi, G., *Proc. Roy. Soc.* **144B,** 100 (1955).

Bovine Serum Ultrafiltrate as a General Standard in Clinical Chemical Analysis

Abraham Saifer and Claire Deutscher

A NUMBER OF PREVIOUS SURVEYS (1, 2, 3, 4) have indicated wide discrepancies of analytic results obtained by various clinical laboratories on the same standard samples. These surveys emphasized the prevailing need for substantial improvement in this field of analysis. One attempt to do this has been through the publication of *Standard Methods of Clinical Chemistry* by the American Association of Clinical Chemists (5) so that the same procedure may be used by many laboratories. A second approach to narrowing the spread of results obtained by various laboratories is the use of primary standards. While such standards are available from the National Bureau of Standards for commercial materials, there has as yet been no comparable program for the clinical chemistry laboratory. A number of standards *for single constituents* are available from commercial sources, but there is still a great need for a *multiple, stable general standard* accurately analyzed and containing nearly all the constituents found in human blood. This need has been highlighted recently by Goeckel (6).

Recently as a result of developments in industrial ultrafiltration technics[1] it has become possible to prepare large batches of a protein- and sterol-free blood serum ultrafiltrate. This material contains all the filterable biologic constituents in the proportions usually found in human blood serum. It seemed desirable, therefore, to investigate its potentialities as a multiple general laboratory standard for clinical chemical

From the Department of Biochemistry and the Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

The authors wish to acknowledge their debt to the personnel of the laboratories listed in Table 1 without whose aid this statistical study could not have been completed. We are grateful also to Miss Renee Eisner and Miss Ethel Cohen for typing and editing the manuscript.

Received for publication July 14, 1955.

[1] The ultrafiltrate used in this study and description of the method of preparation was made available to the authors by Dr. C. F. Hiskey of International Biochemical Corporation, Brooklyn, N. Y.

analyses other than those for proteins and sterols. That study is the subject of this paper.

METHOD OF PREPARATION

Approximately equal quantities of whole blood were drawn on 2 successive days at one of the local abattoirs. This blood was collected after the throat of the animal was slashed and the esophagus clamped shut to prevent contamination by gastric juices. In all a total of 556 L. was drawn, defibrinated and then refrigerated in 5-gallon tin cans until ready for use.

Separation of Blood Cells

Separation of the blood cells was begun immediately after the last portion of blood had been collected. Unfortunately the length of time for which the whole blood was held prior to removal of cells resulted in an appreciable loss of glucose. The cells were removed by centrifuging in a motor-driven Sharples Supercentrifuge. Operation was set with a feed rate of 2.76 L./min. which divided into discharge rates of 1.6 L./min. of serum and 1.16 L./min. of the formed elements. A total of 318 L. of pale-red serum was collected in a stainless-steel tank. After its volume was noted, it was protected from organisms by the addition of 0.02% and 0.18% by weight of propyl and methyl esters, respectively, of *p*-hydroxy benzoic acid.

Filtration

The serum was next transferred to stainless-steel pressure tanks connected by stainless-steel tubing to a production molecular filter. This unit has a membranous filtration tube of 3500 $cm.^2$ area supported by a cotton braid to withstand the 100 lb. working pressure. It started operation at a filtration rate slightly in excess of 2000 ml./hr. and fell to about one half that value in 72 hours of operation. The serum proteins were withdrawn intermittently as a seven- to tenfold concentrate. The filter was reactivated by washing with distilled water. In both reactivations it returned to its original rate. Approximately twenty liters of serum were lost in the reactivations and holdups, and another 35 L. discarded as protein concentrate, and 50 L. lost accidentally, leaving a total production of 278 L. of ultrafiltrate, leaving a net of 213 L. for vialing.

Assurance of Uniformity

The filtrate which had been collected in glass carboys was now pooled, the *p*H adjusted to 7.3 with carbon dioxide, and then given a final

sterilization by filtration. From this point a completely aseptic technic was used in the vialing operation. A total of about 4700 vials was produced. The first vial and every ninety-seventh vial thereafter was withdrawn and given a code number used in the standardization program. In addition the batch was tested for sterility and for evidences of bacterial metabolism—that is, pyrogens—according to procedures outlined in the U. S. Pharmacopeia. Both tests were negative. The entire lot was then put into storage at 4°.

Each of the 48 vials withdrawn for standardization was coded in such a manner that the analyst would be unaware of its position in the vialing operation. Ten of these vials scattered uniformly throughout the batch, including the first and last vial, were analyzed for their CO_2 content. These analyses had identical values within the normal limits of error—that is, 58.3 ± 0.5 volume %. There was no trend of the data in relation to vialing position. It was, therefore, concluded that the batch was uniform throughout and, therefore, ready for standardization.

PRELIMINARY STUDY

Prior to the present large-scale survey, a preliminary study involving only 6 laboratories had been made in order to assess the suitability of bovine serum ultrafiltrate as a general clinical laboratory standard. This study attempted to evaluate in relation to the accuracy of the results obtained such variants as the keeping qualities of the sterile ultrafiltrate under conditions of ordinary refrigeration, the effect of freezing and thawing the standard samples, the effect of mailing samples to various laboratories without refrigeration, the continued bacterial sterility of the sample, and the uniformity of samples from bottle to bottle. These trial runs indicated good time stability of the samples under the variety of conditions as listed. Repeated analysis of samples of this trial batch over a two-year period demonstrated that there were no significant changes in the sugar, CO_2 content, and sodium and potassium values.

PRESENT SURVEY

As a result of this small-scale survey we were encouraged to undertake a larger survey involving 17 laboratories scattered throughout the United States. No special precautions were taken in mailing these samples. The results obtained in the survey verify the earlier ones as to the time-stability factor of the bovine serum ultrafiltrate under a variety of storage and handling conditions.

In conducting the larger survey ultrafiltrate samples were sent out to 30 different laboratories, of which 17 performed the required duplicate analyses on some or all of the constituents listed in Table 2. The following instructions were sent out with each sample:

1. Except for sugar, calcium, and uric acid, for which the ultrafiltrate should be used in double amounts, the ultrafiltrate should be treated exactly as if it were serum, including the simulated preparation of the protein-free filtrate.
2. The sample should preferably be stored in a refrigerator when not in use, and warmed to room temperature prior to its utilization.
3. Aliquots for sugar, urea nitrogen, and CO_2 analysis should be withdrawn with a sterile syringe when required.

RESULTS

The laboratories involved in this survey, the participating biochemists, and the various methods used by these laboratories in the determination of each constituent are listed in Table 1. It should be noted, however, that not all laboratories reported the requested information with respect to the method, the type of instrument, the quantity of material (whether the method was micro or macro), and the final dilution of the sample. The results for the 10 most commonly reported constituents are shown in Table 2. The mean values and standard deviations for all 17 laboratories were calculated for each constituent and those which fell outside ± 2 S.D. were dropped as being experimentally erroneous. The means and S.D.'s were now recalculated and a new range set at ± 1 S.D. As can be seen in Table 2, except for sugar and creatinine the mean values for both sets of calculations remained virtually the same. Therefore, these values can be accepted as *statistically* correct.

In our opinion an acceptable range would be ± 1 S.D. from these mean values. It was found that two thirds of the reported results fell within these limits. Other constituents reported by too few laboratories to warrant statistical analysis are listed at the bottom of Table 2. The ultrafiltrate was shown qualitatively to contain most of the amino acids commonly found in protein hydrolysates (7).

Among these analyses an attempt was made to ascertain whether there were differences due to the type of instrument used, the procedure employed, and the reproducibility of duplicate runs. In general the variation of results obtained between laboratories for the same constituent varied so widely, even when the same procedure was employed, as

Table 1. Clinical Chemical Methods Used by Participating Laboratories for the Determination of the Constituents of Bovine Serum Ultrafiltrates

Hospital	*Supervisor*	*References for methods used*
1. Jewish Chronic Disease	M. Zymaris	13, 16, 23, 25, 26, 30, 34, 38, 45, 48, 49
2. Queens General	I. Gubernick	11, 15, 21, 25, 26, 31, 34, 39, 43, 48
3. New York	E. Russ	11, 18, 21, 25, 26, 31, 34, 42, 44, 48
4. Bellevue	A. Greenstein	8, 15, 21, 27, 31, 34, 38, 44, 48, 49
5. Jewish (Brooklyn)	A. Sobel	8, 19, 22, 26, 31, 34, 46, 48, 49, 50, 51, 52
6. St. Luke's (NYC)	S. J. Ilka	13, 21, 26, 31, 34, 38, 48
7. V.A. (Bronx)	B. Klein	14, 15, 28, 32, 34, 38, 45, 48
8. Maimonides (Brooklyn)	N. Weissman	14, 15, 21, 27, 31, 34, 38, 46, 48, 49
9. Beth-El	R. S. Wayne	13, 16, 20, 33, 36, 38, 44, 48
10. Mayo Clinic (Minn.)	M. H. Power	8, 19, 22, 26, 34, 42, 48, 50, 51
11. D. C. General (Washington, D. C.)	M. Reiner	8, 17, 21, 27, 31, 35, 40, 43, 48
12. Rochester General (N. Y.)	H. H. Rosenthal	8, 20, 26, 31, 34, 38, 44, 48
13. Stamford (Conn.)	W. R. C. Golden	8, 15, 20, 27, 32, 36, 38, 43, 48
14. Harlem	M. McKenna	8, 15, 20, 24, 29, 34, 37, 44, 48
15. Mt. Sinai (N.Y.C.)	J. Carr	8, 18, 21, 24, 31, 34, 39, 43, 48
16. Montefiore (Bronx)	E. J. Baumann	8, 18, 20, 29, 34, 44, 48
17. Jewish Memorial (N.Y.C.)	D. Mishkind	8, 15, 21, 27, 32, 34, 38, 44, 48

Table 2. Statistical Data Obtained from the Analysis of Bovine Serum Ultrafiltrate Samples Performed in Duplicate by Seventeen Clinical Chemical Laboratories

Constituent	*No. hospitals*	*Original mean and standard deviation*	*Adjusted mean and standard deviation*	*Acceptable Range*	*Units*
Sugar	15	36.5 ± 4.4	35.8 ± 3.7	32.1–39.5	mg./100 ml.
Urea N	14	11.1 ± 1.5	11.4 ± 0.8	10.6–12.2	mg./100 ml.
Creatinine	16	1.8 ± 0.4	1.9 ± 0.2	1.7–2.1	mg./100 ml.
Uric acid	15	1.0 ± 0.3	1.0 ± 0.2	0.8–1.2	mg./100 ml.
Chloride	17	105.3 ± 2.6	104.9 ± 1.8	103.1–105.7	mEq./L.
CO_2 comb. power[a]	16	57.7 ± 2.6	58.3 ± 2.2	56.1–60.5	vol. %
Phosphorus	13	7.9 ± 0.7	7.8 ± 0.5	7.3–8.3	mg./100 ml.
Calcium	13	5.8 ± 0.5	5.8 ± 0.5	5.3–6.3	mg./100 ml.
Potassium	17	7.44 ± 0.23	7.45 ± 0.16	7.29–7.61	mEq./L.
Sodium	17	140.0 ± 3.4	140.0 ± 1.8	138.2–141.8	mEq./L.

Other constituents: N.P.N. (mg./100 ml.)—24.2; 29.5; 25.3; 21.2. Amino acid N (mg./100 ml.)—5.8; 6.1. Magnesium (mg./100 ml.)—2.2; 1.4. Citric acid (mg./100 ml.)—3.6.

[a] Millimols/liter $= \dfrac{\text{Vol. \%}}{2.23}$

It would be more correct to express concentrations of biological constituents in terms of millimoles per liter instead of mg./100 ml.]

to render impossible the analysis of such data. At most, several generalizations can be made:

1. The reproducibility between reported duplicate results for the same sample was surprisingly good.
2. The smallest percentage errors were obtained for the electrolytes such as Na, K (flame photometry), CO_2 combining power (gasometric), and chlorides (titrimetric), with the largest percentage errors falling among the colorimetric methods such as used for sugar, urea nitrogen, creatinine, uric acid, and phosphorus.
3. The Brown (27) procedure for uric acid seemed to give consistently higher results than did the Folin (25, 26) or the Benedict (24) method.
4. No consistent differences were found between methods for urea nitrogen using nesslerization as compared to aeration-titration procedures, or between sugar (reducing substance) values using tungstic acid filtrates as compared to those using zinc filtrates, the latter similarity being due to the lack of protein removal in the preparatory process.

CONCLUSIONS

This survey has shown some facts of considerable interest to the clinical chemist.

First, it is evident that bovine blood serum ultrafiltrate constitutes one of the best general standards so far developed for clinical chemical control analysis. It is a stable product that can be prepared in large lots and kept for relatively long periods of time by storage in a refrigerator. Such a standard will be extremely useful in clinical chemical laboratories in the standardization of their technics and as an aid in the elimination of systematic errors of analysis and in routine laboratory control. On the other hand, the absence of serum proteins and sterols precludes the use of this standard for the analysis of these constituents.

The second fact that emerges from this survey is that errors in clinical chemical analysis still occur. The finding that individual results from selected laboratories have been in error, imposes the obligation of periodic control checkups by the chemist charged with the supervision of the laboratory.

Finally, a general standard of this sort permits an exhaustive comparison of different methods for any given constituent without fear of variation from sample to sample.

REFERENCES

1. Belk, W. P., and Sunderman, F. W., *Am. J. Clin. Path.* **17,** 853 (1947).
2. Snavely, J. G., and Golden, W. R. C., *Connecticut State Med. J.* **13,** 190 (1949).

3. Snavely, J. G., and Golden, W. R. C., *Connecticut State Med. J.* **15**, 667 (1951).
4. Snavely, J. G., Golden, W. R. C., and Cooper, A. B., *Connecticut State Med. J.* **16**, 894 (1952).
5. Reiner, M. (Ed.), *Standard Methods of Clinical Chemistry*, New York, Acad. Press, 1953, Vol. 1.
6. Goeckel, H. J., *Clinical Chemist* **6**, 61 (1954).
7. Oreskes, I., and Saifer, A., *Anal. Chem.* **27**, 854 (1955).

Sugar (Reducing Substances)

8. Folin, O., and Wu, H., *J. Biol. Chem.* **41**, 367 (1920).
9. Benedict, S. R., *J. Biol. Chem.* **76**, 457 (1928).
10. Benedict, S. R., *J. Biol. Chem.* **82**, 369 (1929).
11. Benedict, S. R., *J. Biol. Chem.* **83**, 165 (1929).
12. Benedict, S. R., *J. Biol. Chem.* **92**, 141 (1931).
13. Nelson, N., *J. Biol. Chem.* **153**, 375 (1944).
14. Somogyi, M., *J. Biol. Chem.* **160**, 62 (1945).

Urea Nitrogen

Colorimetric

15. Karr, W. G., *J. Lab. Clin. Med.* **9**, 329 (1924).
16. Hughes, J., and Saifer, A., *J. Lab. Clin. Med.* **27**, 391 (1941).
17. Reiner, M. (Ed.), *Standard Methods of Clinical Chemistry*, New York, Acad. Press, 1953, Vol. 1, p. 118.

Aeration-titrimetric

18. Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.* **11**, 211 (1914); **24**, 117 (1916).
19. Sobel, A. E., Mayer, A. M., and Gottfried, S. P., *J. Biol. Chem.* **156**, 355 (1944).

Creatinine

20. Folin, O., *J. Biol. Chem.* **17**, 469 (1914).
21. Folin, O., and Wu, H., *J. Biol. Chem.* **38**, 81 (1919).
22. Wu, H., *J. Biol. Chem.* **51**, 21 (1921).
23. Peters, J. P., *J. Biol. Chem.* **146**, 179 (1942).

Uric Acid

24. Benedict, S. R., and Behre, J., *J. Biol. Chem.* **92**, 161 (1931).
25. Folin, O., *J. Biol. Chem.* **101**, 111 (1933).
26. Folin, O., *J. Biol. Chem.* **106**, 311 (1934).
27. Brown, H., *J. Biol. Chem.* **158**, 60 (1945).
28. Block, W. D., and Geib, N. C., *J. Biol. Chem.* **168**, 747 (1947).

Chlorides (Titrimetric)

29. Van Slyke, D. D., *J. Biol. Chem.* **58**, 523 (1923–24).
30. Saifer, A., and Hughes, J., *J. Biol. Chem.* **129**, 273 (1939).
31. Schales, O., and Schales, S. S., *J. Biol. Chem.* **140**, 879 (1941).
32. Van Slyke, D. D., Hiller, A., and Plazin, J., *J. Biol. Chem.* **167**, 107 (1947).
33. Franco, V., and Klein, B., *J. Lab. Clin. Med.*, **37**, 950 (1951).

Carbon Dioxide

34. Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.* **19**, 211 (1914); *See also* Reiner, M. (Ed.): *Standard Methods of Clinical Chemistry*, New York, Acad. Press, 1953, Vol. 1, p. 23.
35. Van Slyke, D. D., Stillman, E., and Cullen, G. E., *J. Biol. Chem.* **38**, 167 (1919); *See also* Reiner, M. (Ed.): *Standard Methods of Clinical Chemistry*, New York, Acad. Press, 1953, Vol. 1, p. 19.
36. Natelson, S., *Am. J. Clin. Pathol.* **21**, 1153 (1951).

Phosphorus

37. Benedict, S. R., and Theis, R. C., *J. Biol. Chem.* **61**, 63 (1924).
38. Fisk, C. H., and SubbaRow, Y., *J. Biol. Chem.* **66**, 375 (1925).
39. Kuttner, T. T., and Cohen, H. R., *J. Biol. Chem.* **75**, 517 (1927).
40. Gomori, G., *J. Lab. Clin. Med.* **27**, 955 (1941–42).
41. Shinowara, G. Y., Jones, L. M., and Rhinehart, H. L., *J. Biol. Chem.* **142**, 921 (1942).
42. Taussky, H., and Shorr, E., *J. Biol. Chem.* **202**, 675 (1953).

Calcium

43. Kramer, B., and Tisdale, F. F., *J. Biol. Chem.* **47**, 475 (1921).
44. Clark, E. P., and Collip, J. B., *J. Biol. Chem.* **63**, 461 (1925).
45. Sobel, A. E., and Sklersky, S., *J. Biol. Chem.* **122**, 665 (1937–38).
46. Sobel, A. E., and Sobel, B. A., *J. Biol. Chem.* **129**, 721 (1939).
47. Van Slyke, D. D., and Hiller, A., *J. Biol. Chem.* **167**, 107 (1947).

Sodium and Potassium (Flame Photometry)

48. Hald, P. M., *J. Biol. Chem.* **167**, 499 (1947).
49. Natelson, S., *Am. J. Clin. Pathol.* **20**, 463 (1950).
50. Zak, B., Mosher, R. E., and Boyle, A. J., *Am. J. Clin. Pathol.* **23**, 60 (1953).
51. Reiner, M. (Ed.), *Standard Methods of Clinical Chemistry*, New York, Acad. Press, 1953, Vol. 1, p. 102.

Non-Protein Nitrogen

52. MacFate, R. P., Cohn, C., Eichelberger, L., and Cooper, J. A. D., *Am. J. Clin. Pathol.* **24**, 511 (1954).

Amino Acid Nitrogen

53. Frame, E. G., Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.* **149**, 255 (1943).

Magnesium

54. Orange, M., and Rhein, H. C., *J. Biol. Chem.* **189**, 379 (1951).

Citric Acid

55. Natelson, S., Lugovoy, J., and Pincus, J. B., *J. Biol. Chem.* **170**, 597 (1947).

Chemical Assay of Diethylstilbestrol

R. L. Dryer

THE CHEMICAL ASSAY of diethylstilbestrol in extremely small amounts has not been previously accomplished in a satisfactory manner. The ultraviolet absorption of diethylstilbestrol has been proposed as a method (1), but this fails in the presence of other absorbing substances in tissue extracts. In addition, the absorption curve lacks any sharply defined band. Measurement of the phenolic function (2) suffers from a similar lack of specificity, while methods based on nitration (3, 4, 5) lack sensitivity and specificity. Dingemanse (6) was the first to propose the use of antimony pentachloride as a reagent for diethylstilbestrol, and this reagent has been adopted by Warren, Goulden, and Robinson (7) and by Jones and Deatherage (8). A sensitivity of 0.002 mg. has been claimed for the antimony pentachloride reaction but our experience with it has not been as fortunate, owing to rather high blank optical densities produced by unknown chromogens and to a day-to-day variation of optical densities of standard solutions. We have therefore sought a different approach to this problem.

Diethylstilbestrol reacts with bromine under various conditions to form colored compounds or complexes (9, 10), similar to the behavior of certain 17-α-hydroxysteroids (11). Cocking (12) recently described two reactions of this type, involving diethylstilbestrol, which appeared to be of promise in the analytic sense. We have examined one of these in some detail, and present herewith a chemical assay based on Cocking's observations. The proposed method seems to be quite specific, fairly sensitive, and applicable to the assay of diethylstilbestrol in tissue extracts.

From the Clinical Biochemistry Laboratory of the Department of Biochemistry, College of Medicine, State University of Iowa, Iowa City.

This work was supported in part by Mattox and Moore, Inc., Indianapolis, Ind.
Received for publication July 16, 1955.

REAGENTS

1. Bromine reagent, 1% (v/v) in glacial acetic acid.
2. Acetic acid, glacial.
3. 95% Ethyl alcohol.
4. Ether, peroxide free.
5. Acetone, freshly redistilled.
6. 2.5N Sodium hydroxide.
7. Diethylstilbestrol Standard, 0.1 mg. per ml. in acetic acid.

This stock solution may be used as long as it remains colorless. Solutions tinged with yellow have undergone oxidation and do not react with bromine in the normal manner.

PREPARATION OF TISSUE EXTRACTS

Appropriate samples of tissue are cut in small pieces and homogenized with 15–20 volumes of a 1:1 mixture of acetone and ether in a Waring Blendor equipped with a micro grinder. For soft tissues such as liver a modified Latapie mill may be used with equal success. The homogenate is clarified by filtration through Whatman No. 3 paper previously wet with the solvent mixture. The residue on the paper is again washed twice with small volumes of the solvents, and the combined filtrate and washings are adjusted to a convenient volume, depending on the expected stilbestrol content. Aliquots which contain approximately 0.1 mg. of diethylstilbestrol are transferred to test tubes or aluminum evaporating dishes[1] and evaporated just to dryness on a steam bath.

The residues are treated with 5 ml. of ether and transferred to separatory funnels. The ethereal solutions are treated with 1 ml. of 2.5N NaOH, stoppered, and shaken vigorously. The two phases are separated and the ether is discarded. The alkaline solution is washed twice with successive 5 ml. portions of ether; these are also discarded. Acidify by slow addition of 1 ml. of 5N H_2SO_4. Cool under a cold tap, and add 5 ml. ether. Vigorous shaking transfers the diethylstilbestrol back into the organic phase. The ether layer is separated and evaporated to dryness in preparation for the colorimetric assay. To insure complete separation of the two phases we have found that separatory funnels with capillary stopcocks are most helpful[2] and have the added advantage of fitting into a 50-ml. centrifuge cup for breaking the emulsion which forms in an occasional sample.

[1] A. H. Thomas Co., Philadelphia, Pa., No. 4537.
[2] Scientific Glass Apparatus Co., Bloomfield, N. J., No. R4100

COLORIMETRIC ASSAY

The dried residues obtained from the tissue extracts are dissolved in 1 ml. of acetic acid. Each series of analyses should also include a blank of 1 ml. acetic acid and a standard of diethylstilbestrol in acetic acid. Add 0.1 ml. of the bromine-acetic acid reagent to all the tubes, and place them in a vigorously boiling water bath for exactly 2 minutes. Remove the tubes promptly and cool under the tap. The contents of all the tubes should have a pale yellow color, indicating free bromine. Since the final colored complex does not form in the absence of free bromine there is no point in continuing with colorless solutions. The cause of decolorization is either an excessive ratio of diethylstilbestrol to bromine reagent or inadequate separation of nonspecific bromine absorbing substances.

After the tubes are cooled, add 2 ml. of 95% alcohol, and mix. Finally, add 3 ml. of water and mix well. As the water is added a rose or violet color is produced which has a maximum optical density at 500 mμ. The color intensity should be determined 1 minute after the water is added.

RESULTS

When known quantities of diethylstilbestrol are submitted to the colorimetric procedure just described, the optical density plot indicates that Beer's law is obeyed at least within the range of 0.005–0.125 mg. of the estrogen (Fig. 1) when measurements are corrected for the blank. The exact density depends markedly on the time of heating (Fig. 2), so that close attention should be paid to control of the stated 2 minute interval. This was arbitrarily chosen to give the widest useful range of optical densities. Figure 3 is the transmission curve (corrected) of the final colored product. The single peak is broad enough to permit measurements with the 35 mμ light band width of the Coleman Jr. spectrophotometer, using 9 x 76 mm. cuvets. Unfortunately, the color is not stable indefinitely; it fades in the manner depicted by Fig. 4. For this reason the optical densities should be measured at a definite time interval after the addition of the water which produces the color. We have chosen 1 minute for this interval, but other times would presumably be satisfactory. In spite of the definite dependence on the presence of free bromine the rate of color fading does not seem to follow the classical first-order mechanism, at least in the time interval studied.

The transfer of diethylstilbestrol from ether to alkali is essentially quantitative. Samples of 1 mg. of the estrogen were dissolved in ether and shaken with sodium hydroxide as described. No trace of color was found when the entire volume of ether was evaporated and subjected to

5. Gottlieb, S., *J. Am. Pharm. Assoc.* **36**, 379 (1947).
6. Dingemanse, E., *Nature*, 145, 825 (1940).
7. Warren, F. L., Goulden, F., and Robinson, A. M., *Biochem. J.*, **42**, 151 (1948).
8. Jones, O., and Deatherage, F. E., *Food Research*, **18**, 30 (1953).
9. Unpublished data.
10. Osol, A., and Farrar, G. E., *Dispensatory of the United States of America*, vol. I, 363, J. B. Lippincott, Philadelphia (1950).
11. Miescher, K., and Kagi, H., *Helv. Chim. Acta*, 32, 761 (1949).
12. Cocking, T. T., *Analyst*, **68**, 144 (1943).

Observations on Electrophoretic Analysis of Normal Human Serum

Robert S. Gordon, Jr.

With the installation of a new electrophoresis apparatus in this laboratory, it appeared desirable, as a preliminary study, to analyze a number of normal human sera. A summary of the significant findings is presented in this communication.

MATERIALS AND METHODS

The Aminco Model B electrophoresis apparatus was used; the quantitative results reported were derived exclusively from its interferometric optical system. Sera to be analyzed were obtained from normal employees at this institution. Their ages ranged from 18 to 54 with a mean of 31, both sexes were represented, and all but two were white. A tourniquet was applied briefly to facilitate venipuncture, but extensive stasis was avoided. Subjects were in general not fasting, blood being taken between the hours of 9:00 and 11:00 A.M. The samples were diluted sixfold and dialyzed thoroughly against a sodium diethylbarbiturate buffer of ionic strength 0.1 M and *p*H 8.7 ± .05. In the experiments reported in Table 2, a pair of serum samples was dialyzed together in one large vessel of buffer, and run simultaneously in the two cells of the electrophoresis apparatus. The cells were connected in series, and operated from a single power supply. The volumes of the cell channels were carefully calibrated with mercury, and a reference mark for mobility measurements was placed on the cell itself. Each run lasted 3 hours at a current flow of 15 mA. Every assembled cell was tested for electrical leaks; none had a resistance of less than 2×10^7 ohms to ground.

From the Laboratory of Metabolism, National Heart Institute, National Institutes of Health, Bethesda Md.

Received for publication August 25, 1955.

Interpretative Criteria

In the interpretation of the photographs, the region between minima in the refractive gradient was considered to define a component. Mobility measurements refer to the position of the median fringe of the component in question. The conductivity of the buffer, measured at 1.1°, was used in the mobility calculations. Values of $\Delta n/\Delta W$ given by Armstrong, Budka, and Morrison (1) were used to calculate the relative contribution of each component to the total protein, the ascending limb pattern being used exclusively. Total protein was likewise calculated from the electrophoretic pattern which, if the boundary anomaly be included, measures the total refractive index difference between buffer and protein solution. When it became apparent that the total protein values were rather higher than expected, gravimetric dry weight determinations, employing 24-hour air drying at 105°, were instituted. As applied to whole serum, this procedure appears justified by the data tabulated by Armstrong, Budka, Morrison, and Hasson (2). Excellent agreement with the values derived from the electrophoretic patterns was obtained, but the values given in Table 1 represent the refractometric data exclusively.

RESULTS AND COMMENT

Protein Compositions

Table 1 summarizes the protein compositions observed in the analyses of 37 normal sera.

The findings should be compared with those reported by Reiner, Fenichel, and Stern (3), who published a comparable investigation as well as an excellent summary of older data. It will be noted that the relative concentrations reported here agree well with the earlier ones, whereas the absolute concentrations appear to be significantly higher. This discrepancy is believed to be due largely to the difference in the

Table 1. Relative and Absolute Protein Compositions of Normal Human Sera

Component	*Relative conc. (% of total)*		*Absolute conc. (Gm.% dry wt.)*	
	Mean	*S.D.*	*Mean*	*S.D.*
Albumin	56.3	2.6	4.47	.25
Globulin				
α_1	5.7	0.7	.45	.06
α_2	9.3	1.3	.74	.11
β	14.7	1.6	1.17	.14
γ	14.1	2.6	1.12	.24
Total			7.95	.40

method used to obtain total protein. Reiner *et al.* (3) employed Kjeldahl nitrogen × 6.25 (4) as total protein, whereas, as Armstrong *et al.* (2) point out, nitrogen × 6.73 gives a better estimate of the total dry weight of plasma proteins. Because of the similarity of the refractive index increment per gram of dry weight among the various plasma fractions, it would appear that dry weight is the more reliable unit in which to report concentrations. If the average values given by Reiner *et al.* (3) be multiplied by 1.08, they are only slightly lower than the values reported here.

Mobility of Albumin Component

Table 2 records the mobility of the albumin component for each of the 26 sera run in pairs. The constancy of the mobility of the albumin component which was revealed by the paired mobility runs was surprising, especially in view of the wide range of albumin mobilities that have been reported in the literature. The 26 mobility runs in Table 2, plus 3 other single runs on which mobility data are satisfactory, yield an average mobility value of -6.92×10^{-5} cm.^{2}/volt sec., with a standard deviation of $\pm .04 \times 10^{-5}$ in the ascending limb, and, in the descending limb, -6.65×10^{-5} cm.^{2}/volt sec. with a standard deviation of $\pm .04 \times 10^{-5}$. The descending mobility value agrees well with that given by Longsworth and Jacobsen (5) for bovine albumin in a similar buffer. In the ascending limb, the observed difference between the two individuals in a pair experiment was as great as $.04 \times 10^{-5}$ in only 1 out of 13 pairs. It is therefore apparent that the experimental variables of buffer *p*H and conductivity, as well as total current flow, which are eliminated from consideration

Table 2. Mobilities of Albumin Component in Normal Human Sera

Cell #1			Cell #2				
Donor	$-\mu \times 10^5$		Donor	$-\mu \times 10^5$		$\Delta\mu \times 10^5$	
	Asc.	Desc.		Asc.	Desc.	Asc.	Desc.
D.F.	7.00	6.69	E.M.	6.98	6.73	.02	−.04
R.A.	6.93	6.69	W.R.	6.93	6.70	.00	−.01
J.C.	6.92	6.67	C.K.	6.91	6.62	.01	.05
E.E.	6.94	6.67	E.P.	6.94	6.69	.00	−.02
O.G.	6.89	6.62	E.L.	6.92	6.67	−.03	−.05
F.B.	6.90	6.66	H.C.	6.94	6.69	−.04	−.03
J.L.	6.94	6.68	T.Q.	6.92	6.64	.02	.04
M.B.	6.92	6.66	W.P.	6.91	6.64	.01	.02
D.H.	6.93	6.63	W.H.	6.91	6.58	.02	.05
M.B.	6.97	6.71	W.W.	6.95	6.67	.02	.04
C.D.	6.82	6.55	A.S.	6.83	6.56	−.01	−.01
B.H.	6.91	6.60	M.V.	6.93	6.60	−.02	.00
I.J.	6.90	6.62	J.R.	6.87	6.62	.03	.00

in the comparison of the two members of a pair, are responsible for a large part of the observed variation in mobility as usually measured. The variability that remains can be explained by errors inherent in measuring the photographic plates, by the possible effects on albumin mobility of bound ions which do not dialyze (such as the higher fatty acids), and by the likelihood that quantitatively unimportant protein species whose electrophoretic mobility differs slightly from that of true albumin may affect to a small but variable extent the electrophoretic mobility of the albumin component.

There is no need to postulate an inherent variability in the mobility of the pure albumin protein contained in the sera of different individuals. The possibility remains, however, that abnormal albumins, analogous to the various types of abnormal hemoglobin, exist in small segments of the population and that no such individual happened to be sampled in this limited study. A number of pathologic sera have, however, been tested in this laboratory by electrophoresis simultaneously with a normal serum, and to date no significantly abnormal albumin mobility has been encountered.

The mobility of the descending albumin component appears to be somewhat more variable in the paired experiments, so that the use of descending limb mobilities for the empirical comparison of one and another serum is less satisfactory. This effect is to be anticipated, since in the descending limb the albumin is moving in a dilute solution of the other serum proteins, which may influence albumin mobility by their effects on conductivity, *p*H, and viscosity, even if no direct interaction in the albumin occurs. These effects may not be equal in each of two sera, particularly if one is a pathologic specimen. In the ascending limb, a dependence of observed mobility only on albumin concentration may be predicted, but the effect should be small relative to experimental errors. A scattergram prepared by plotting $\Delta\mu$ from Table 2 against the difference in albumin concentration in the two cells showed no convincing correlation. A similar scattergram of albumin mobility against buffer *p*H yielded no correlation, as the predicted effects were again small compared to anticipated experimental errors.

REFERENCES

1. Armstrong, S., Budka, M., and Morrison, K., *J. Am. Chem. Soc.* **69**, 416 (1947).
2. Armstrong, S. Budka, M., Morrison, K., and Hasson, M., *J. Am. Chem. Soc.* **69**, 1747 (1947).
3. Reiner, M. J., Fenichel, R., and Stern, K., *Acta Haematologica* **3**, 203 (1950).
4. Reiner, M., personal communication to the author.
5. Longsworth, L. G., and Jacobsen, C. F., *J. Phys. Coll. Chem.* **53**, 129 (1949).

Serum Globulin Fractions in Chronic Rheumatic Diseases

An Electrophoretic Study

Harold B. Salt

A STUDY OF THE SERUM protein patterns in patients with chronic rheumatic diseases has already been reported in an earlier publication (1). In that work the proteins were separated by a salting-out technic and the fractions estimated by the biuret reaction. The results confirmed that hyperglobulinemia is a frequent finding in chronic rheumatic conditions, being often accompanied also, though in less degree, by hypoalbuminemia. The serum globulin was obtained in three fractions, and when hyperglobulinemia was noted the increments were located chiefly in the euglobulin and pseudoglobulin I fractions.

EARLIER METHODS

Through recent years, many of the older analytical methods for serum proteins have been critically re-examined, and improved technics have been developed. The presently available microanalytical methods have recently been reviewed (2). It is now generally regarded as unsatisfactory to attempt the detailed separation of serum protein into characteristic fractions by salting-out methods, though certain technics, whereby substantially the total globulins are precipitated and albumin alone is left remaining in solution, may be used with fair confidence. For this purpose concentrations of neutral salts, higher than those classically employed, are necessary; for example, 28% sodium sulfite instead of 21% as formerly used. Because of this, many of the earlier studies on serum albumin

From the Biochemical Laboratory, The Royal Infirmary, Worcester, England.
Present address: The Children's Hospital, Birmingham, England.

I gratefully record the continual interest taken in this work by the late Dr. H. L. Milles, who had care of the patients; and acknowledge with pleasure the assistance of Mrs. P. Limrick in all the practical work.
Received for publication June 7, 1955.

and globulin concentrations must be interpreted with caution, as the albumin values tend to be too high because of the inclusion of some of the globulin moiety, and the globulin values correspondingly too low.

It thus appeared that the previous studies (1) could well be revised in order to establish truer values for the serum albumin and total globulin levels in patients with chronic rheumatism. At the same time, it was hoped to explore the use of an ammonium sulfate-sodium chloride reagent recently advocated (3) for the specific precipitation of the serum γ-globulin fraction, and to determine this (as well as the albumin and total protein) directly by an improved application of the biuret reaction. As will be seen later, this salting-out method for the separation of the γ-globulin fraction proved, in our experience, to be distinctly limited in its applicability.

For the fractionation of the serum globulins in more complete detail the technic of microelectrophoretic separation on absorbent paper was adopted as the best means now available.

PRESENT INVESTIGATIONS

The purpose of this work was to apply the microelectrophoretic method to the routine study of patients with chronic rheumatic conditions, and to define with adequate precision the nature and extent of the changes in serum protein patterns which develop characteristically, though non-specifically, in those conditions.

Material

One adult patient with mildly active rheumatoid arthritis was selected for long-term study and samples of her serum were examined serially throughout a period of 30 weeks.

Besides this, a random series of blood-serum samples was examined, being derived from a sequence of 26 adult patients whose conditions included subacute (active) and chronic (inactive) rheumatoid arthritis, osteoarthritis, and fibrositis. These patients were unselected, except for ensuring that a whole range of cases, from the mildest to the most severe, was included. The analytic results obtained for the 26 patients' sera were collected into two groups, characterized by the existence or absence of hyperglobulinemia, and the data evaluated statistically.

A more detailed study was made of the abnormal globulin patterns in the hyperglobulinemic sera; and the possibilities of detecting hyper-γ-globulinemia by a simple (salting-out) technic were also explored.

Analytic Methods

Serum total protein was estimated absorptiometrically by an adaptation (3) of the biuret reaction, except that only 1.5% Rochelle salt was included in the biuret reagent.

For estimations of the albumin fraction, the total globulins were precipitated by the use of 28% sodium sulfite (3). Serum globulin concentrations were obtained by the difference between the total protein and albumin values.

A serum fraction, designated γ-globulin, was also determined by the biuret reaction; the fraction being obtained by a salting-out method (3), modified (4) in that only 3.0% NaCl instead of 4.0% was included in the ammonium sulfate-sodium chloride precipitating reagent.

The electrophoretic separations of albumin and of the serum globulin into four fractions were done in a horizontal apparatus similar to that of Grassmann *et al.* (5), using Whatman No. 100 paper, 0.06M barbital buffer at *p*H 8.6, and current conditions of 120 v. for 16 hours.

After the electrophoretic separation was completed, the papers were dried for 30 minutes at 100° and the proteins stained with bromophenol blue, using the aqueous staining reagent and subsequent acid-aqueous washing procedure described by Kunkel and Tiselius (6).

For quantitative estimation of the several proteins, each paper strip was cut transversely into five parts containing respectively the albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin. A sixth protein-free segment was also cut to serve as a blank. The length of each segment was noted, so that appropriate blank-corrections could be applied, and the dye present in each was eluted into 10 ml. 0.01N sodium hydroxide solution during a period of exactly 30 minutes. The concentrations of dye were then determined in terms of net extinction (optical density) values.

It was known that there is usually a disparity in the amounts of bromophenol blue bound by any given weight of albumin and of globulin, and that the estimated amounts of adsorbed dye do not necessarily provide correct estimations of the several proteins in every case. In consequence, it was found best to accept as correct the values obtained by chemical means for albumin and for total globulin; and to partition the total globulin into four fractions, in proportion to the amounts of bromophenol blue bound respectively by the four electrophoretically separated globulin components.

RESULTS

Table 1 shows the serum protein values, determined on 9 occasions during a period of 30 weeks, in a patient with early rheumatoid arthritis.

Table 1. SERIAL DETERMINATIONS IN 1 PATIENT AND NORMAL VALUES FOR SERUM PROTEINS AND GLOBULIN FRACTIONS

Weeks		*Proteins* (Gm./100 ml. serum)			*Globulin fractions* (Gm./100 ml. serum)			
Total	*Interval*	*Total*	*Albumin*	*Globulin*	α_1-	α_2-	β-	γ-
0	0	7.84	4.26	3.58	0.38	0.98	1.06	1.16
6	6	7.14	4.37	2.77	0.27	0.74	0.81	0.95
8	2	7.40	4.90	2.50	0.26	0.63	0.67	0.94
12	4	7.20	4.50	2.70	0.23	0.77	0.79	0.91
14	2	7.60	4.40	3.20	0.32	0.76	1.12	1.00
18	4	6.90	4.10	2.80	0.34	0.65	0.81	1.00
22	4	7.10	4.30	2.80	0.32	0.77	0.83	0.88
26	4	7.20	4.30	2.90	0.33	0.70	0.81	1.06
30	4	6.80	4.50	2.30	0.29	0.54	0.65	0.82
Mean		7.24	4.40	2.84	0.30	0.73	0.84	0.97
Usual Normal Range		6.3–8.3	3.6–5.0	2.3–3.7	0.2–0.4	0.5–1.0	0.5–1.1	0.6–1.5

On every occasion, her serum protein concentrations were found to be within the normal range, a finding consistent with the fact that her clinical condition was only mild in degree, though essentially unchanged despite appropriate treatment. In cases of greater severity, a variety of abnormal serum protein patterns was observed.

In the whole group of 26 patients whose serum protein patterns were investigated, 12 were found to have serum globulin concentrations within normal limits (below 3.7 Gm./100 ml.) and 14 to have varying degrees of hyperglobulinemia. The analytic results were divided on this basis into two subgroups and a summary of the data is presented in Table 2. Mean values with standard deviations are shown for each protein fraction, together with the values for t and P (7) which furnish a statistical evaluation of the magnitude and significance of the differences between the means.

It is evident from Table 2 that there is a significant difference ($P <$ 0.05) between the two subgroups in respect to every protein fraction studied. The sera having normal concentrations of total globulin were also substantially normal in albumin, in total protein, and in each of the globulin fractions; whereas in the subgroup having hyperglobulinemia, the sera were frequently low in albumin and elevated in total protein, and the increase in total globulin was due to increments in one or more of all of the globulin fractions.

Table 3 is a frequency diagram showing the incidence of elevated globulin fractions in 11 sera characterized by definite hyperglobulinemia. The

Table 2. SUMMARY OF ANALYTICAL DATA FROM SERA OF 26 PATIENTS

Serum proteins (Gm./100 ml.)	*Serum globulin*								*Statistical evaluation of the differences between the means*	
	Below 3.7 Gm./100 ml. (12 patients)				*Above 3.7 Gm./100 ml.* (14 patients)					
	Range of values found	*Mean value*	*S.D.*	*Mean ± 2 S.D.*	*Range of values found*	*Mean value*	*S.D.*	*Mean ± 2 S.D.*	t	P
Globulin (total)	2.20–3.60	3.00	0.48	2.04–3.96	3.81– 7.60	4.77	1.03	2.71–6.83	5.48	<0.01
Globulin fractions[a]										
α_1-	0.26–0.38	0.32	0.04	0.24–0.40	0.27– 0.47	0.38	0.06	0.26–0.50	2.93	0.01
α_2-	0.54–0.88	0.73	0.10	0.53–0.93	0.72– 1.99	1.03	0.32	0.39–1.67	3.11	<0.01
β-	0.50–0.98	0.77	0.15	0.47–1.07	0.71– 1.30	1.04	0.16	0.72–1.36	4.48	<0.01
γ-	0.62–1.61	1.17	0.29	0.59–1.75	1.51– 3.91	2.32	0.81	0.70–3.94	4.64	<0.01
Albumin	3.50–5.20	4.35	0.52	3.31–5.39	2.50– 4.50	3.41	0.58	2.25–4.57	4.30	<0.01
Total protein	6.70–8.10	7.35	0.46	6.43–8.27	7.30–10.50	8.18	0.84	6.50–9.86	3.05	<0.01

[a] Separated electrophoretically.

Table 3. Incidence of Elevated Globulin Fractions in Sera of 11 Patients with Hyperglobulinemia[a]

Globulin fraction found to be elevated[b]	Patterns of elevated globulin fractions											Frequency of elevation of each fraction
γ-	+	+	+	+	+	+	+	+	+	+	..	10
α_2-	+	+	+	+	+	+	+	..	..	..	+	8
β-	+	+	+	..	..	..	..	..	..	+	+	5
α_1-	+	..	..	+	+	..	..	..	..	..	..	3
Frequency of pattern among the 11 sera	1	2		2		2		2		1	1	

[a] Total globulin 4.10 to 7.60 Gm./100 ml. serum.

[b] Concentration greater than the value "Mean + 2 S.D.," as shown in Table 2 for the group of 12 patients without hyperglobulinemia.

Table 4. Comparison of Values by Two Methods for Serum γ-Globulin

Number of patients	Serum γ-globulin (Gm./100 ml. serum)								Statistical evaluation of the difference between the means	
	By electrophoretic separation				By ammonium sulfate-sodium chloride precipitation					
	Range of values found	Mean value	S.D.	Mean ± 2 S.D.	Range of values found	Mean value	S.D.	Mean ± 2 S.D.	t	P
10[a]	0.62–1.34	1.09	0.23	0.63–1.55	0.46–1.30	1.01	0.25	0.51–1.51	0.73	0.5
16[b]	1.51–3.91	2.23	0.80	0.63–3.83	1.00–1.90	1.51	0.24	1.03–1.99	3.46	<0.01

[a] Electrophoretically separated gamma-globulin within normal limits.

[b]Electrophoretically separated gamma-globulin above the normal range.

11 sera (out of 14 with total globulin levels above the normal range) were those in which the total globulin exceeded the value "mean + 2 S.D.," as found for the subgroup without hyperglobulinemia; and elevation of any fraction was recorded by (+) only when the concentration of that fraction likewise exceeded the corresponding "mean + 2 S.D." as found in the subgroup without hyperglobulinemia. Borderline values were thus avoided and only definite elevations in the serum globulin fractions taken into account in constructing the globulin patterns.

It is evident from Table 3 that the hyperglobulinemia of chronic rheumatic disease was most frequently found to be due to increments in the γ-globulin fraction and that these increments were often accompanied also by increased concentrations of α_2-globulin. Less frequently, increased amounts of β-globulin were found and in some cases the α_1 fraction was also elevated.

The frequent finding of increased concentrations of electrophoretic serum γ-globulin led to speculation that the determination of the γ fraction, after separation by the salting-out technic, might also provide useful information. Accordingly, the protein fraction precipitated by the ammonium sulfate-sodium chloride reagent was determined in each of the 26 serum samples simultaneously with the electrophoretic studies.

The results were divided into two groups: (a) a group of 10 wherein the electrophoretically separated serum γ-globulin concentrations were entirely within normal limits and (b) a group of 16 wherein the concentrations were elevated above the normal range. A summary of the results is presented in Table 4.

It is evident from the values and calculated data (Table 4) that in the series with normal γ-globulin concentrations the electrophoretic and salting-out methods of separation yielded results closely in agreement, the apparent small differences being statistically insignificant (P much greater than 0.05). In contrast to that finding, in the group of 16 sera with elevated electrophoretic γ-globulin concentrations (and of which 14 had increased total globulin levels that had to be accounted for) the values for the γ-globulin fraction, as obtained by ammonium sulfate-sodium chloride precipitation, were profoundly deficient. The differences between the mean values for the two series of estimations is large and statistically significant (P much less than 0.05); moreover, an examination of the 16 individual paired analyses revealed that the salting-out method yielded results lower in every case than the corresponding values obtained electrophoretically.

DISCUSSION

Many of the earlier investigations into the serum protein changes in rheumatoid arthritis have been critically summarized (8, 9). Some apparent discrepancies between the results of different investigators may be ascribed to variations in the moving-boundary electrophoretic technic used, also perhaps to some uncertainty as to the scope of normal values; moreover, different patterns of abnormality may appear during successive phases of the disease process. Recent investigations (10, 11, 12, 13) have substantially confirmed the earlier findings, and the work of others (14, 15, 16) has added extra details to our knowledge.

The technic of microelectrophoretic separation of serum proteins on absorbent paper differs appreciably from the moving-boundary method of electrophoretic analysis and this leads to differences between the results obtained by the two procedures (17).

In one investigation by a paper-electrophoretic technic, Hunt and Trew (18) claim to have demonstrated increased relative fibrinogen and γ-globulin levels with correspondingly lowered albumin fractions in the plasma of arthritic patients, but failed to show significant alterations of α- and β-globulins. However, they regarded their technic merely as a screening method and one that proved to be only partially satisfactory.

In the work presently described, the interfering effects of fibrinogen encountered by Hunt and Trew (18) were avoided by choosing serum as the material for study, and restricting the observations to albumin and the four globulin fractions adequately separated by the technic employed. The results agree substantially with those hitherto obtained by the more laborious moving-boundary method; though certain special details of the electrophoretic patterns delineated by that procedure (14, 15, 16) are doubtless indefinable by paper electrophoretic technics.

It may be mentioned, however, that in some instances detailed cutting of the electrophoretic paper strips (40 parts) was also done and curves were drawn for the sera of several of the most severely affected patients. In one patient with depleted serum albumin and greatly increased α_2-, β-, and γ-globulins, "split-peaks" were found initially for both the beta- and gamma-components. Ten weeks later, when the patient was appreciably better, his serum still showed a large increase in γ-globulin and a slight increase in β-globulin, but without "split-peaks"; thirteen weeks after this, when the patient was greatly improved, his serum proteins were normal except for a very slight increase in the γ-globulin fraction. At the present stage of experience, apart from accepting "split-peaks" as evidence of heterogeneity of the proteins, it seems best to withhold any attempt at further interpretation.

The failure of the ammonium sulfate-sodium chloride reagent to precipitate all of the γ-globulin from sera having abnormally high concentrations of this protein cannot be adequately explained. The method provided approximately correct results from normal sera, so that merely technical faults seem to be unlikely. Possibly the method was so devised originally as to be adequate for those abnormalities of serum γ-globulin that occur in other (for example, liver) diseases, wherein the whole serum-protein structure may differ from that in chronic rheumatic disorders, and by its nature influence favorably the complete precipitation of the γ-globulin. However that may be, there is small loss in discarding the salting-out technic for γ-globulin. Serum samples can first be classified readily according to the existence or absence of (total) hyperglobulinemia, and then their whole protein pattern can be delineated adequately by applying the technic of microelectrophoresis in the manner described.

CONCLUSIONS

The experiments here reported indicate that a study of the serum proteins can provide useful information in cases of chronic rheumatic disease. The more severe cases are likely to show some depletion in serum albumin and an increase of greater magnitude in the concentration of serum globulin.

As a means of demonstrating increases in the γ-globulin fraction in patients with rheumatic disorders, the salting-out technic using an ammonium sulfate-sodium chloride reagent is unreliable.

The technic of microelectrophoresis on paper excellently reveals any abnormalities in the pattern of the globulins. Such abnormalities vary in nature and extent and may involve one or more of the four globulin components.

SUMMARY

Investigations into the serum protein patterns that occur in chronic rheumatic diseases, formerly made by salt-fractionation methods, are now revised with the aid of the superior technic of microelectrophoretic separation.

Using the microelectrophoretic method, supplemented by a single salt-fractionation procedure, the concentrations of albumin, α_1-globulin, α_2-globulin, β-globulin and γ-globulin in the sera of 26 patients variously affected by chronic rheumatic diseases were determined.

Normal protein patterns were found in all the 12 sera with normal total globulin content, whereas variously abnormal protein patterns were found in all of the 14 hyperglobulinemic sera.

The hyperglobulinemia of chronic rheumatic diseases was found most frequently to be due to increments in γ-globulin, often accompanied also by increases in α_2-globulin. Less frequently, increased amounts of β-globulin were found, and in some cases an elevation of α_1-globulin. These abnormalities were all detectable electrophoretically, whereas the method for the salting-out of γ-globulin was unsatisfactory.

REFERENCES

1. Salt, H. B., *Ann. Rheumatic Diseases* **10,** 46 (1951).
2. Salt, H. B., *Analyst* **78,** 4 (1953).
3. Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F., *Am. J. Clin. Path.* **18,** 723 (1948).
4. de la Huerga, J., and Popper, H., *J. Lab. Clin. Med.* **35,** 459 (1950).
5. Grassmann, W., Hannig, K., and Knedel, M., *Deut. med. Wochschr.* **76,** 333 (1951).
6. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.* **35,** 89 (1951).
7. Fisher, R. A., *Statistical Methods for Research Workers* (ed. 8). Edinburgh, Scotland, Oliver and Boyd, (1941), P. 120.
8. Gutman, A. B., *Advances in Protein Chem.* **4,** 155 (1948).
9. Marrack, J. R., and Hoch, H., *J. Clin. Path.* **2,** 161 (1949).
10. Svartz, N., and Olhagen, B., *Acta Med. Scand.*, **130,** Suppl. 206, 456 (1948).
11. Routh, J. I., and Paul, W. D., *Arch. Phys. Med.* **31,** 511 (1950).
12. Leinwand, I., *J. Lab. Clin. Med.* **37,** 532 (1951).
13. Ropes, M. W., Perlmann, G. E., Kaufman, D., and Bauer, W., *J. Clin. Invest.* **33,** 311 (1954).
14. Wallis, A. D., *Ann. Internal Med.* **32,** 63 (1950).
15. Reid, A. F., Pike, R. M., Sulkin, S. E., and Coggeshall, H. C., *J. Lab. Clin. Med.*, **37,** 264 (1951).
16. Layani, F., Bengui, A., and de Mende, S., *Semaine Hôp. Paris* **28,** 3221 (1952).
17. Cooper, G. R., and Mandel, E. E., *J. Lab. Clin. Med.* **44,** 636 (1954).
18. Hunt, T. E., and Trew, J. A., *Ann. Rheumatic Diseases* **13,** 201 (1954).

PRESIDENT'S ADDRESS

THE AMERICAN ASSOCIATION OF CLINICAL CHEMISTS is now in the seventh year of its existence, and this is our seventh annual meeting. Though I am not a numerologist, I can't refrain from talking a little about the magical number seven.

From ancient times on, seven has been regarded by many as having a mystical significance. There are the seven lean years and the seven fat years in the Bible, there is the seven-branched candlestick which Titus carried to Rome after the capture of Jerusalem; and Rome herself is built on Seven Hills. There are the Seven Wonders of the World and ships are sailing the Seven Seas. We hear about Snow White and the Seven Dwarfs, about seven-league boots, and about the seven national saints. A week has seven days . . . this list could be continued for quite a while.

As scientists we are curious, of course, to know the reason for the magic of seven. The best explanation seems to be that the mystical meaning of this number was strengthened and perhaps even suggested by its use in astrology, in which the seven planets played a prominent part. Our precursors, the alchemists, I might add, had seven metals corresponding to these seven planets—lead for Saturn, copper for Venus, iron for Mars, and so on.

Jaques in Shakespeare's *"As You Like It"* speaks of seven ages of man:

> All the world's a stage,
> And all the men and women merely players;
> They have their exits and their entrances;
> And one man in his time plays many parts,
> His acts being seven ages.

Jaques goes on to analyze these distinctive periods of growth from infancy to senescence. Applying this analysis ot the American Association of Clinical Chemists, one can say that our association is now leaving infancy behind and is entering the second stage. One must admit that it is a promising child that is getting into the schoolboy age, bringing along not

Delivered at the Seventh Annual Meeting of the American Association of Clinical Chemists, September 15, 1955, Minneapolis, Minn.

only a book (Volume I of *Standard Methods*), but also the first volume of his own journal, CLINICAL CHEMISTRY.

I wish there were on hand a seventh daughter of a seventh daughter of a seventh son, since these are known to have the rare gift of seeing into the future. But since I have not found one, we must make our own predictions without benefit of supernatural assistance.

First of all, I believe, it should not be too difficult to produce during the next year one, or possibly two, additional volumes of *Standard Methods in Clinical Chemistry*. Volume III is in the planning stage. It will include a number of toxicologic procedures and should be particularly welcome, since it will eliminate a last-minute rush to the library to scan the widely scattered toxicologic literature when the sudden need for an emergency test arises. Volume I of *Standard Methods* was published over 2 years ago, in 1953, and some of you feel that succeeding volumes are overdue. Nothing good, of course, can come of haste, but while I am opposed to pressure as much as anybody, I feel it is not unreasonable to schedule the appearance of new volumes as regular annual events. I hope that a volume dealing with ultra-micro methods will soon become a reality, especially since we have talent in our midst well qualified to write on this subject. The use of ultra-micro methods is essential for the proper care of premature infants. Their value is not restricted, however, to this field of pediatrics. There are, for example, unexplored areas of the blood chemistry of small animals, which could only benefit from the availability of ultra-micro methods. All of us have encountered the dilemma of a battery of tests to be done with insufficient blood. Ultra-micro methods will be of great value in such instances.

Turning to our journal, CLINICAL CHEMISTRY, I believe we can be optimistic about its future. The Board of Editors have stated as their objectives: "to help provide answers to the many problems facing those engaged in clinical chemistry; to create and maintain standards of scientific research and writing that will reflect honorably upon our profession; and to provide a continuing forum for discussion of the scientific, technical, and professional problems of all members of our profession." In looking over Volume 1, one can perceive the effort expended by the Board of Editors in the realization of these objectives. It should be pointed out, however, that this is your journal and that all of us must feel responsible for it and participate in it, each to the best of his ability.

There are many other activities and responsibilities of the American Association of Clinical Chemists which could be covered in this annual address. I will restrict myself, however, to a few words about only one

other subject—the relationship between the profession of clinical chemistry and the medical profession.

Gone is the time when the medicine man went out into the moonlit night to collect rare herbs and prepared mysterious concoctions for the treatment of his patients. Drugs now come in little packages from the pharmacist's shelf and the practicing physician would not dream of retiring to his laboratory to synthesize a batch of acetylsalicylic acid for tomorrow's headaches. He gladly turns this task over to the pharmaceutical chemists, confident that the bottle bought at the pharmacy will contain aspirin if so labeled. The physician welcomes the help and assistance given by the pharmaceutical chemist. He certainly does not claim that the preparation of drugs for the treatment of disease constitutes the practice of medicine. This claim is not made, despite the fact that the material prepared by the pharmaceutical chemist is introduced into the system of a living human being.

Turning now to the clinical chemist, we all know, of course, that he does not prepare materials to be given to a patient orally, subcutaneously, intramuscularly, or intravenously. The clinical chemist is concerned with the analysis of material coming out of the patient no longer part of his living system, and not with the preparation of substances going in. Let me add hastily that in my 20 years of close contact with the medical profession I have never met an individual who has even pondered the possibility that the task performed by the clinical chemist might be construed as constituting the practice of medicine.

The last century has seen an enormous development in all fields of science, and it is, unfortunately, not possible any more for any one man to master all disciplines equally well. We are in a period of specialization and the united efforts of many individuals in many fields are needed to render the best possible service in any area of our complex civilization. This includes services to the patient. To ensure best medical care, it is essential that internists, surgeons, bacteriologists, radiologists, pathologists, clinical chemists, and many others cooperate and contribute their part. The problems of public health are too serious a matter to tolerate quibbling with self-appointed little demigods about ill-advised monopolistic tendencies. What matters is the welfare of the patient, and each of the various specialists participating in the attack on a medical problem must contribute his services in that segment of endeavor in which by virtue of his training and his experience he is qualified to work. If his training has been in chemistry and if he has learned to carry out analytical determinations on body fluids accurately and reliably, he is a clinical chemist. One becomes a clinical chemist through years of study and

through additional years of practical training. One does not become a clinical chemist by purchasing a book on laboratory methods or because one can collect and apply analytical data for the solution of a medical problem. A course in general biochemistry is not sufficient preparation for the practice of clinical chemistry. I took a course once in general surgery, which was duly credited. Nevertheless, I am not obsessed by the delusion that I am qualified to perform even the simplest surgical procedure. Let me repeat that best medical care requires the cooperation of many specialists, each serving in his field of training.

In the complex society in which we live, it is necessary to have legislation designed to protect the public welfare. An old German proverb says: "*Schuster bleib' bei deinen Leisten,*" and if the cobbler doesn't agree to stay with his lasts, one might have to regulate his activities in the public interest through laws and legislation. Applying this thought to clinical chemistry, one could visualize legislation restricting the practice of clinical chemistry to those qualified by training and experience to practice it. A signature on a laboratory report, for example, certifying that a serum calcium concentration reported is a true and reliable value, should be the signature of a clinical chemist who is responsible for the result. A rubber-stamp impression of the name of somebody unfamiliar with the determination of calcium is also visible, but it is worthless as far as responsibility in a legal sense is concerned. I have heard of places where the clinical chemist is embarrassed while carrying out his professional duties by the existence of anachronistic legislation. We all know that we are not living in Utopia and it is hardly worthwhile, I believe, to waste words on the remnants of such medieval ignorance. When Faust reflected upon the modes of human endeavor, he came to the conclusion that it wasn't words but work and accomplishment that made a man's life worthwhile.

In this spirit the clinical chemist can look into the future with confidence. He must show again and again in his daily contacts with the medical profession that he is doing his share through accurate and reliable work, as a member of a team united in efforts to help the patient. With such an attitude of service and devotion, recognition is inevitable. Among the seven gifts of the spirit, there are three which are of particular value—knowledge, wisdom, and understanding. You must have a thorough knowledge of your field, so that your services are truly valuable. You must acquire wisdom, so that you can see beyond the walls of your laboratory and become a constructive element in our culture. Finally you must have an understanding of human nature, since such an understanding is absolutely essential to establish a true spirit of cooperation.

OTTO SCHALES

the Clinical Chemist

EDITORIAL

THE International Congress of Clinical Chemistry will be held in New York City during the week of September 9, 1956. The American Association of Clinical Chemists, at the suggestion of the International Federation of Clinical Chemists, has undertaken the responsibility of organizing and sponsoring this Congress. The success or failure of the project will depend, of course, on the cooperation given to it by the clinical chemists throughout the world.

This Association has held seven annual national conventions, the first before the Association was one year in existence. These annual meetings have all been successful and were enjoyed by the participants. One of these even had an international aspect, as it was held in conjunction with the Diamond Jubilee of the American Chemical Society in 1951. At that time two overseas clinical chemists were awarded honorary membership in the Association, and other scientists had an opportunity to visit with their American colleagues.

The pending Congress, however, will be the first international meeting to be held in the United States devoted exclusively to clinical chemistry. A most interesting program is being prepared and will consist of many scientific sessions as well as scientific and industrial exhibits. The persons concerned with the organization of the Congress have already prepared the groundwork for an intensive schedule. The numerous details are being

seriously considered and various committees are being set up to handle them. Each local section of the Association will be encouraged to support this Congress if only by encouraging their membership to contribute papers and participate in the sessions.

This is not the appropriate time to review the past activities of the Association. Suffice it to say that every previous major undertaking has been accomplished creditably. These successes have been due in part to the individuals who have given unselfishly of their time and efforts, but also, and of greater importance, they have been due to the wholehearted support of the membership that has molded the American Association of Clinical Chemists into a useful organization and a tribute to its profession. This year we will have the added responsibility as hosts to our Latin-American and overseas colleagues—The International Congress of Clinical Chemistry requires the same support to assure its success.

M. M. F.

INTERNATIONAL CONGRESS

The American Association of Clinical Chemists is sponsoring an International Congress of Clinical Chemistry, to be held in New York City from September 9 to 14, 1956.

The program of the Congress will have as its principal topic "The Significance of Metabolic Systems in Clinical Chemistry." Morning sessions will be devoted to reviews of selected subjects by invited speakers, mainly from overseas; at the afternoon sessions will be presented the contributed papers. There will be both scientific and commercial exhibits, and entertainment will include trips to points of interest in and around New York, as well as a Congress dinner.

Abstracts of papers should be sent to the Chairman of the Scientific Program, Dr. Harry Sobotka, the Mount Sinai Hospital, New York 29, N. Y., before May 15, 1956. Arrangements with regard to exhibits should be made with Dr. Charles L. Fox, Chairman of the Scientific Exhibits Committee, New York Medical College, New York, also before May 15.

A more detailed announcement will be made later. Meanwhile, all those

interested in contributing to the success of the Congress are urged to get in touch with the chairmen of the appropriate committees, which include in addition to those named above, the following:

Chairman of the Congress: Albert E. Sobel, Jewish Hospital of Brooklyn, Brooklyn, N. Y.

Secretary: John G. Reinhold, University of Pennsylvania, Philadelphia, Pa.

Housing and Hospitality Committee, Metropolitan New York Section: Chairman, Julius Carr, Mount Sinai Hospital, New York, N. Y.; Secretary, A. Saifer, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

News Service Committee: Chairman, Joseph Samachson, Montefiore Hospital, Bronx, N. Y.

Considerable interest has already been shown in the Congress. Approximately 150 contributed papers are expected, and the plans to invite guests from abroad are maturing rapidly. As the number of sessions devoted to contributed papers will be limited, all those who wish to contribute are urged to get in touch with Doctor Sobotka at the earliest possible date.

SCIENTIFIC PROGRAM

The program for the scientific sessions of the International Congress is taking form. The present plans include four morning and three afternoon periods. The morning sessions will be devoted to specific subjects centering around one or two review papers for which prominent speakers will be invited. The morning programs will not be divided into parallel sessions, a procedure which has proved its value at the Biochemical Congress in Paris, as it permits maximum attendance. The subjects will be in the area of "Metabolic Systems in Clinical Chemistry." Some contributed papers that pertain to the specific subject will be grouped around these nuclei. The afternoon sessions will be devoted to the remainder of the contributed papers and may be divided into parallel sessions if the material is sufficiently abundant.

Abstracts of 200 words are required, to be submitted by May 15, 1956. Two mailings of the official abstract forms will be made, one in January and one in April. Arrangements are being made with the editor and publishers of CLINICAL CHEMISTRY to have the abstracts published as a supplement to the journal and to be distributed to the Congress members at the time of registration.

The Scientific Program Committee, still incomplete, consists of Harry Sobotka, Chairman, Reginald Archibald, Louis B. Dotti, Monroe E. Freeman, Joseph I. Routh, and Warren M. Sperry.

NOMINATING COMMITTEE

The 1955 Nominating Committee recommends to the members of the American Association of Clinical Chemists the following list of candidates to serve as officers during the year beginning July 1, 1956:

President:

Robert M. Hill
Denver, Colo.

Vice-President:
Joseph I. Routh,
Iowa City, Iowa
National Secretary:
Max M. Friedman
New York, N. Y.
National Treasurer:
Louis B. Dotti,
New York, N. Y.

and for members of the Executive Committee:

Emmett B. Carmichael, Birmingham, Ala.
W. E. Cornatzer, Grand Forks, N. D.
George R. Kingsley, Los Angeles, Calif.
Martin Rubin, Washington, D. C.
Otto Schales, New Orleans, La.

The Nominating Committee finds that the growth of the Association has increased the duties and responsibilities of the National Secretary and the National Treasurer to such an extent that it would be most reluctant to urge their re-election unless clerical assistance is made available to them. It therefore requests, by unanimous vote, that the Executive Committee authorize the expenditure of funds for such assistants without delay.

John G. Reinhold, *Chairman*
Miriam Reiner
Joseph I. Routh
Albert E. Sobel
Harry Sobotka
Warren M. Sperry
Arnold G. Ware

NOMINATIONS FOR ERNST BISCHOFF AWARD

The membership is again urged to submit nominations for the 1956 Ernst Bischoff Award. The Executive Committee has agreed to simplify the process of nomination. The résumé to be submitted need consist only of a summary outlining the accomplishments of the nominee and a brief statement in support of the recommendation. A complete bibliography is not required. Nominations should be submitted in triplicate and sent to Dr. Joseph H. Roe, 1335 H Street, N.W., Washington, D. C. Those received on or before March 31, 1956, will be considered for the 1956 Award, which will be presented this year during the International Congress in New York City.

CHANGE OF ADDRESS

Members are requested to notify the National Secretary of any change of address. This is essential if the Association files are to be kept in order. Nonmember subscribers should notify the publisher of such changes.

MEMBERSHIP SCROLLS

Members and associate members can order a membership scroll, suitable for framing, by sending $4.00 to Dr. Louis B. Dotti, National Treasurer, St. Luke's Hospital, New York 25, N. Y. Applicants should indicate how they wish their names inscribed, with academic degrees. The scrolls are distributed at one time during each year, usually following the annual meetings in the fall.

ARREARS IN ANNUAL DUES

Members in arrears in payment of annual dues will be dropped from the roster unless such arrears are paid in full by March 1, 1956. Subscriptions

to Clinical Chemistry will be automatically withdrawn.

Individuals dropped in this manner assume the liability for current dues and may be reinstated at some later date only through the process of new membership. Resignations will be accepted up to the above cut-off date.

REPORTS FROM THE SECTIONS

Boston

At the October meeting the following new officers were elected:

Chairman—Eli Dubinsky

Vice-Chairman—William Cohen

Secretary-Treasurer—Esther E. Thomas

Note

The listing of the Board of Editors, Departments, and Advisory Board of Clinical Chemistry on page xii of this issue is to be pasted on the back of the volume title page provided in the December 1955 issue of the Journal.

BOOK REVIEWS

Techniques in Clinical Chemistry

FREDERICK N. BULLOCK. Bristol, England, John Wright and Sons Ltd.; Baltimore, The Williams and Wilkins Co., 1954, 171 pp., $4.00

The past two years have seen a number of excellent books on clinical chemistry from authors in Great Britain. Upon receipt of this present work the reviewer envisioned another good work from that country. The book was a decided disappointment.

The author states in the preface that the book originated from notes made during a two-year period spent in the British West Indies teaching student technicians, and that it is intended to supplement the more exhaustive manuals that are available. Though the reviewer is not familiar with the scientific background of Dr. Bullock's "student technicians," one is led to believe from the opening chapter, which describes the "Fundamentals," that the laboratory experience of these students does not encompass a course in chemistry at more than secondary school level. Yet the methodology that the author has chosen to include, of which more than one half are volumetric and iodometric procedures, would seem to require a degree of analytical skill for the standardization of solutions and preparation for the analyses far above the capabilities of student technicians.

In preparation for his choice of methodology, Bullock does go into some detail on the apparatus employed in volumetric analysis. He does not discuss photoelectric colorimetry, "for it is felt that whilst electrophotometry has a place in research investigations for estimating very minute concentrations of certain substances, it has a very limited value in routine diagnostic work." Though methods for use with the Dubosq type of colorimeter are included, the author does not devote any space to the correct usage of this type of instrument.

The descriptions of the procedures are illustrated with the chemical equations for the reactions. For the oxidation-reduction reactions the outdated "oxygen release" is used rather than "ion-election" equations. For the more important determinations colorimetric, volumetric, and iodometric procedures are described in good detail.

Though limited space does not allow a critical review of all procedures, one very obvious statement under "Carbon Dioxide Combining Power of Plasma" should be corrected. In the description of the technic, the reader is told to place the plasma in a separatory funnel and "fill with alveolar air or with carbon dioxide from a cylinder. Shake the funnel in such a way that the plasma is thoroughly mixed with the gas, as much as possible is thereby dissolved. Two minutes of such treatment is usually sufficient to saturate the plasma with carbon dioxide." The instructions should present the correct procedure to insure the

total chemical combination of the carbon dioxide at *normal alveolar pressure* rather than the physical saturation of the gas in the liquid.

The appendix contains tables of the British specifications for volumetric glassware. There are, according to American chemical standards, a number of errors in the "Table of Normality of Concentrated Acids and Bases." Concentrated HCl is approximately 12N rather than 10N. Concentrated NH_4OH is approximately 15N rather than 20N. In giving the amounts of NaOH and KOH needed to make 1 liter of approximately normal solution, the author has increased the calculated amounts by 2 Gm., presumably to take into account some of the carbonate content.

This book cannot be recommended as a useful work in clinical chemistry, other than for illustration that there are analytic procedures other than colorimetric in this specialty.

Metropolitan Hospital, New York, N. Y. HAROLD D. APPLETON

The Roger Adams Symposium

New York, John Wiley & Sons, Inc., 1955, 140 pp., $3.75

To the never-ending flow of tributes accorded Dr. Roger Adams, is added *The Roger Adams Symposium.* This volume consists of six papers presented in Dr. Adams' honor at the Symposium held on September 3 and 4, 1954, at the University of Illinois.

Products of Dr. Adams' inspired teaching, the six contributors to "The Roger Adams Symposium" are all eminent scientists in their own right. Their papers reflect the directions of their original thinking as well as the attainments of the man they esteem.

In his introductory remarks, carried over from the symposium as the opening chapter in this book, Dr. Ernest H. Volwiler reviews the already well-known accomplishments of this great man of chemistry. Dr. Wallace R. Brode discusses "Steric Effects in Dyes" and Dr. John R. Johnson deals with "The Structure of Gliotoxin, a Sulfur-Containing Substance."

"The Structure of Nepetalic Acid" is considered by Dr. Samuel M. McElvain. Dr. Ralph L. Shriner's subject is "Chemistry of Flavylium Salts: Reactions with Amines." In the final paper, Dr. Wendell M. Stanley writes on "Some Chemical Studies on Viruses."

The two-day symposium was attended by former students and faculty associates of Roger Adams from all parts of the country. At the same time, Adams' work and actual presence are familiar to people all over the world and made him, so to speak, a community project. In writing of the symposium's success, Dr. Carl S. Marvel therefore states in the book's preface, ". . . its historical significance created a desire to have a record of the Symposium that would be permanent and could be made available to interested persons irrespective of school affiliation. To this end the present volume has been prepared." As a further memento, a full-color photograph of Dr. Adams appears opposite the title page.

Electrochemistry in Biology and Medicine

T. SHEDLOVSKY (Ed.) New York, John Wiley & Sons, Inc., 1955, 369 pp., \$10.50.

This book grew out of a Symposium on Electrochemistry in Biology and Medicine held in New York by The Electrochemical Society in April, 1953. One way in which electrochemistry is of vital importance to biology and to medical science is in providing the basis for many of the laboratory methods and tools used in these fields. As examples, one may consider, among others, the technics used for measuring acidity, oxidation-reduction, ionic mobility, thermodynamic activity, diffusion, dipole moments, and dielectric constant. Another reason for the importance of the role which electrochemistry plays in these fields is based on the fact that all living cells are, in part, electrochemical systems capable of transforming chemical energy and ionic transport into electrical potentials. With appropriate apparatus, such electrical signals may sometimes be utilized to increase our knowledge of the functioning of nerve and muscle or, in a very practical manner, may be used by the phsyician in arriving at a diagnosis. The discussions, from the writings of twenty-three investigators, attempt to cover some of the more important electrochemical aspects concerning membranes, animal and plant cells, biologically important ions, and some limited applications of polarography, electrocardiography, and electroencephalography in the field of medical science.

As the editor of the volume points out, a symposium has the character of a forum, and no author assumes any responsibility for the content of chapters other than his own. Rather than providing an orderly, exhaustive treatise on electrochemistry in biology and medicine, the individual chapters hang but loosely together to form a heterogeneous over-all treatment. The book is not one that would ordinarily be read as a unit or used as a textbook in a university course. Rather, it is likely to be studied by persons who will find something of real interest in only one or two chapters. The level of presentation varies widely; for example, the chapter by Scatchard on transport of ions across charged membranes and the chapter by Schmitt on dynamic negative admittance components in statically stable membranes are presented in a much more exact and mathematical fashion than, say, the presentation by Hitchcock on membrane potentials in the Donnan equilibrium.

One of the useful ends which are served by this book, in the opinion of this reviewer, is to bring together in one volume diverse electrochemical research activities. To the reader, who may be interested primarily in one or two topics of discussion, gentle browsing through the remainder of the book is almost certain to lead to a fruitful cross-fertilization of research ideas. The tone of the book is controversial and it serves in an admirable manner the purpose of highlighting certain regions in the area between physical science and life.

Stritch School of Medicine
Loyola University, Chicago, Ill.

HUGH J. McDONALD

ABSTRACTS

Editor: Ellenmae Viergiver. *Contributors:* Joseph S. Annino, Gladys J. Downey, Clyde A. Dubbs, Alex Kaplan, Margaret M. Kaser, Miriam Reiner, Herbert Thompson

Methodology in determination of cholesterol: A review. B. Zak and M. Ressler (*Wayne University College of Medicine and Wayne County General Hospital, Eloise, Mich.*).

The need for a rapid and accurate method for the determination of cholesterol in blood or plasma has occupied clinical chemists for many years as evidenced by the tremendous number of modifications of the several basic procedures in the literature. The pathologic significance of cholesterol in diagnosis as well as its possible role in the pathogenesis of atherosclerosis and probable role as a precursor of steroidal hormones are cited. The chronologic development of cholesterol methodology and the extraction, saponification, and isolation of cholesterol, color development, and other pertinent factors are critically discussed. The number of variable factors related to the Liebermann-Burchard reaction appear to make the testing of other end points, such as the reaction with ferric chloride or anthrone, or nephelometry a necessity for the development of the most practical and useful method. The authors conclude that among the methods which may meet present-day requirements are those of Pearson, Stern, and McGavick [*J. Clin. Endocrinol. and Metabolism* **12,** 1245 (1952) and *Anal. Chem.* **25,** 813 (1953)] and Zlatkis, Zak, and Boyle [*J. Lab. Clin. Med.* **41,** 486 (1953)], but further verification is necessary before they can be accepted.—*Am. J. Clin. Pathol.* **25,** 433 (1955). (H. E. T.)

Hemoglobin standardizations: Commentary on procedures to insure reliable hemoglobinometry. F. W. Sunderman, B. E. Copeland, R. P. McFate, V. E. Martens, H. S. Naumann, and G. F. Stevens (*Jefferson Medical College, Philadelphia, Pa.*).

The paper "Proposal for the Distribution of a Certified Standard for Use in Hemoglobinometry" by R. Keith Cannan [*Am. J. Clin. Pathol.* **25,** 376 (1955); Clin. Chem. **1,** 151 (1955)] is discussed in detail. The authors believe that the determination of the iron content of blood for standardization of the hemoglobin procedure is practical and adequate in almost every clinical laboratory. From a diagnostic point of view, the routine measurement of hemoglobin as cyanmethemoglobin may actually be disadvantageous unless the results are correlated with oxyhemoglobin measurements, since hemoglobin is most frequently used clinically as an index of the oxygen-carrying capacity of the blood. The

authors also object to the view that the routine use of a reagent containing 52 mg./L. of potassium cyanide constitutes a "negligible hazard."—*Am. J. Clin. Pathol.* **25,** 489 (1955). (H. E. T.)

Application of laboratory controls in clinical chemistry. A. S. Benenson, H. L. Thompson, and M. R. Klugerman (*Second Army Area Medical Laboratory, Fort George G. Meade, Md.*).

The preparation and storage of control serum are described. The control serum which simulates the characteristics of the specimen to be analyzed is run along with each series of unknown samples. A series of clinical specimens is assumed to be in "control" when the value obtained on the control serum is within predetermined allowable limits. Methods of standardizing and estimating precision are presented. The need for a reference standard with each set of unknown samples rather than reliance on a calibration curve is emphasized. The allowable limits for 17 commonly measured blood serum components are given.—*Am. J. Clin. Pathol.* **25,** 575 (1955). (H. E. T.)

Significance of doubly refractile fat bodies in urinary sediment. D. V. Walz and D. C. James (*Veterans Administration Hospital, Iowa City, Iowa*).

Doubly refractile bodies were found in 23 urines from 22 patients out of a total of 3633 urines examined. The bodies were not consistently present in repeated urine examinations from any one patient. The fat bodies did not appear to be characteristic of any specific disease category and their presence was of no aid in differential diagnosis. Doubly refractile lipoid bodies were found more frequently in seriously ill patients, suggesting that their presence is of ominous significance.—*Am. J. Clin. Pathol.* **25,** 598 (1955). (H. E. T.)

Colloidal glass suspensions for use as standards for measurement of thymol turbidity. J. G. Reinhold (*Hospital of the University of Pennsylvania, Philadelphia, Pa.*).

Colloidal glass suspensions are prepared by mechanical agitation of borosilicate glass fragments in distilled water to form a milky suspension. The suspension is diluted 1 to 1 with water and allowed to stand 72 hours. The uppermost 300 ml. are then decanted for use. This suspension provides a superior turbidity standard that is stable for months and has optical absorption properties resembling those that occur in the thymol turbidity test.

Evaluation showed that glass suspensions prepared by several other methods are unsatisfactory, since they do not produce a proper distribution of particle sizes. Added preservatives, with the exception of $HgCl_2$, have no advantage. A neutral *p*H is recommended.

The turbidity produced by the thymol-barbital reagent with serum has

different absorbencies with different photometers. The extent of these differences depends in part on the protein and lipid composition of the serum.—*Anal. Chem.* **27,** 239 (1955). (C. A. D.)

Lipide and protein composition of four fractions accounting for total serum lipoproteins. L. A. Hillyard, C. Entenman, H. Feinberg, and I. L. Chaikoff (*University of California School of Medicine, Berkeley, Calif.*).

Total lipoproteins were separated into four fractions according to specific gravity: A, less than 1.063; B, 1.063 to 1.107; C, 1.107 to 1.220; and D, greater than 1.220. Protein, cholesterol, phospholipide, and triglyceride contents were determined on all four serum fractions in man, dog, rabbit, rat and chicken. There was some similarity between these lipoprotein fractions, A, B, C, and D, and those reported by others, β, α_2, and α_1. The total serum lipoproteins in man varied considerably but the fractions with respect to protein were similar in all species studied.—*J. Biol. Chem.* **214,** 79 (1955). (J. S. A.)

A comparison of routine plasma volume determination methods using radioiodinated human serum albumin and Evans blue dye (T-1824). R. E. Zipf, J. M. Webber, and G. R. Grove (*Miami Valley Hospital, Dayton, Ohio*).

The results of simultaneous plasma volume estimations with radioiodinated human serum albumin and T-1824 dye are presented. The radioisotope method is preferred because of its higher degree of precision and its lesser technical and clinical limitations.—*J. Lab. Clin. Med.* **45,** 800 (1955). (G. J. D.)

The determination of iron in plasma or serum. T. H. Bothwell and B. Mallett (*Radcliffe Infirmary, Oxford, England*).

Previous technics for the estimation of iron in plasma were simplified without loss in accuracy by eliminating the waiting periods after the addition of hydrochloric and trichloroacetic acids, respectively. Recovery experiments with Fe^{59} demonstrated that iron is quantitatively separated from β_1-globulin and measured by using the procedures outlined.—*Biochem. J.* **59,** 599 (1955). (A. K.)

The isolation and estimation of urinary mucoproteins. A. J. Anderson and N. F. Maclagan (*Westminster Medical School, London, England*).

Urinary mucoproteins were adsorbed upon benzoic acid and then heated with acid diphenylamine reagent for 30 minutes at 100°. The intensity of the resulting purple color was measured at 540 mμ. Male subjects excreted more mucoproteins than females. The daily excretion was significantly increased in respiratory infections. *Biochem. J.* **59,** 638 (1955). (A. K.)

A method for the fractionation and measurement of 17-ketosteroids in human plasma. C. J. Migeon and J. E. Plager (*University of Utah College of Medicine, Salt Lake City, Utah*).

A method has been devised for the measurement of dehydroepiandrosterone (DHA) and androsterone in human plasma. The technic consists of the following steps: 1. Ethanol extraction of 25 ml. of plasma; 2. Evaporation of the ethanol extract and solution of the residue in water; 3. Continuous extraction with ether at *p*H 0.8 for 48 hours; 4. Washing of the ether extract with $NaHCO_3$ and water; 5. Chromatography on Florisil columns; 6. Removal of phenols with 1N NaOH; 7. Paper chromatography and elution of paper areas corresponding to DHA and androsterone; and 8. Micro-Zimmermann reaction.

In 15 normal males, the average plasma level of DHA was 450 μg./100 ml., and of androsterone, 18 μg./100 ml. The levels in 8 normal females were not significantly different from those in males.—*J. Clin. Endocrinol. and Metabolism* **15,** 702 (1955). (M. R.)

Nature and transport of the iodinated substances of the blood of normal subjects and of patients with thyroid disease. W. S. Dingledine, R. Pitt-Rivers, and J. B. Stanbury (*Harvard Medical School, Boston, Mass.*).

It is confirmed that thyroxine is transported in the plasma largely by protein with an electrophoretic mobility between the α_1 and α_2 globulins, and to a lesser extent by the albumin fraction. Triiodothyronine in vitro becomes associated with the electrophoretically distinguishable plasma proteins and has a peak concentration similar to that of thyroxine in the inter-alpha zone. No I^{131}-labeled substances other than iodide, thyroxine, and triiodothyronine were found in the sera of 27 patients who received I^{131}. Thirteen of these patients had Graves' disease, 4 had toxic nodular goiter, 2 had nontoxic goiter, and 8 had normal thyroids. These studies furnish no evidence that there are qualitative differences between normal subjects and those with various thyroid disorders in the iodinated components of the blood or in the mechanisms of their transport.—*J. Clin. Endocrinol. and Metabolism* **15,** 724 (1955). (M. R.)

Excretion of amino acids in nephrosis. W. W. Shreeve, M. E. Hutchin, H. A. Harper, C. D. Miller, and P. D. Doolan (*U. S. Naval Hospital, Oakland, Calif.*).

Seventeen natural and 1 unnatural (*d*-methionine) amino acids were determined by microbiologic assay in the blood and urine of 2 adults with nephrosis. The urinary excretion in 1 subject was definitely increased in comparison with the excretion of 14 normal individuals previously studied. In the other subject there was a tendency toward increased excretion, but less marked. After intravenous loading with a mixture of amino acids, the percentage of filtered amino acids determined by the simultaneous measurement of inulin clearance was increased

in both normal and nephrotic subjects, with no consistent differences between them. The blood-amino-acid levels tended to be low in both nephrotic patients, but was significantly below the normal level only for tryptophane and valine and, in 1 patient, for arginine. Administration of ACTH to 2 normal subjects produced no definite changes in amino acid excretion but seemed to cause a moderate decrease in 1 nephrotic individual.—*Proc. Soc. Exp. Biol. Med.* **88,** 510 (1955). (M. M. K.)

Dehydroascorbic acid level in blood of patients suffering from various infectious diseases. B. Chakrabarti and S. Benerjee (*Presidency College, Calcutta, India*).

Dehydroascorbic acid and ascorbic acid were determined in the blood of 28 normal subjects, and before treatment and during convalescence in 25 cases of meningococcal meningitis, 25 cases of tetanus, 23 cases of typhoid fever, 22 cases of acute lobar pneumonia, and 17 cases of tubercular meningitis. The proteins were precipitated from freshly drawn oxalated blood with sulfosalicylic acid and removed by centrifugation and filtration of the centrifugate through absorbent cotton. Ascorbic acid in the filtrate was determined by titration with 2:6-dichlorophenol-indophenol by the method of Menaker and Guerrant [*Ind. Eng. Chem. Anal. Ed.* **10,** 25 (1938)]. Hydrogen sulfide gas was passed through another aliquot of the filtrate to reduce dehydroascorbic acid to ascorbic acid, and the H_2S was removed by a current of nitrogen. The treated filtrate was then titrated to obtain both ascorbic acid and dehydroascorbic acid, and the difference was used as a measure of dehydroascorbic acid. During the acute phase of all the diseases investigated the dehydroascorbic acid was considerably increased above the very low level found in normal subjects, while the ascorbic acid content was decreased. During convalescence these trends were reversed, but normal values were not reached.—*Proc. Soc. Exp. Biol. Med.* **88,** 581 (1955).
(M. M. K.)

Proteinuria following intravenous norepinephrine and epinephrine in man. S. E. King and D. S. Baldwin (*U. S. Army Hospital, Fort Jay, N. Y.*).

After 15 hours of fluid and salt restriction, observations were made on 6 normal male subjects and 6 subjects with intermittent orthostatic proteinuria. Urine samples were collected by indwelling catheter. After a control period, norepinephrine was infused in 10 individuals at a constant rate varying from 15 to 44 μg./min. for 16 to 45 minutes with the rate adjusted to obtain a systolic pressure elevation of 50 to 70 mm. of mercury. Similarly, epinephrine was given at a rate of 15 to 22 μg./min. for 24 to 33 minutes in 4 subjects. Observations were continued from 23 to 54 minutes following the infusions, with blood pressure and pulse rate being determined at 5-minute intervals. Qualitative estimations of urine protein were made by precipitation with sulfosalicylic acid in samples collected at 5-minute intervals during and after the infusions.

Proteinuria was induced by norepinephrine in 4 out of 5 normal subjects and in 4 out of 5 subjects with intermittent proteinuria, and persisted for as long as 50 minutes after discontinuation of the administration of the pressor amine. Epinephrine caused proteinuria for periods up to 48 minutes in 2 normal and in 2 proteinuric subjects.—*Proc. Soc. Exp. Biol. Med.* **88,** 624 (1955).

(M. M. K.)

A rapid clinical method for the determination of calcium in serum and other biological fluids. B. Rehell (*University of Helsinki, Finland*).

Calcium is precipitated from serum as the oxalate. An excess of standard ethylene-diamine-tetraäcetic acid and a buffer solution are added. The mixture is then back-titrated with magnesium chloride solution. The indicator consists of a mixture of eriochromeschwarz T and methyl red. The color change is from green to red and the end point is said to be easily seen. The end point is much more difficult to notice if direct titration is employed or if serum is titrated directly without precipitating the calcium. The method is applicable to 0.1 ml. of serum with an error of about 5 per cent. When 1 ml. of serum is used, the error is about 2 per cent. *Scand. J. Clin. & Lab. Invest.* **6,** 335 (1954). (E. V.)

A chromatographic method for the determination of the three bile pigments in serum. B. H. Billing (*Postgraduate Medical School, London, England*).

A quantitative method has been described for the determination of the three bile pigments, bilirubin and pigments I and II, which give a direct reaction in the van den Bergh test.

Since the pigments could not be eluted quantitatively from the usual type of partition chromatogram, a column was designed (detailed diagram is given) so that after separation of the pigments, the supporting *kieselguhr* could be removed and divided into compartments, each of which contained a pigment. The pigments were then converted to the corresponding azo compound and extracted by shaking with alcoholic diazotized sulfanilic acid.

The procedure requires about 1 ml. of serum and the separation of the pigments takes less than 20 minutes. The method is not applicable to serum levels of less than 5 mg. of bilirubin per 100 ml.

There was no significant difference between the total amount of pigment I and II determined chromatographically and the amount of "direct bilirubin" determined by the method of Malloy and Evelyn [*J. Biol. Chem.* **119,** 481 (1937)]. The prompt reacting "1 minute bilirubin" described by Ducci and Watson [*J. Lab. Clin. Med.* **30,** 293 (1945)] could not be identified with either of the directly reacting bile pigments in serum.—*J. Clin. Pathol.* **8,** 126 (1955).

(E. V.)

Plasma creatinine. M. Ralston (*Postgraduate Medical School, London, England*).

The Lloyd's reagent adsorption procedure was investigated chromatographically. Only one Jaffe-positive spot, identical with that for creatinine, was observed. It is concluded, therefore, that the Lloyd's procedure is specific for creatinine in plasma.—*J. Clin. Pathol.* **8,** 160 (1955). (E. V.)

Serum transaminase as a measure of myocardial necrosis. D. Steinberg and B. H. Ostrow (*National Institutes of Health and George Washington University, Washington, D. C.*).

Transaminase was determined by a modification of the method of Karmen, Wroblewski, and LaDue [*J. Clin. Invest.* **34,** 126 (1955)] in which transaminase activity is coupled with the malic dehydrogenase reaction. Under suitable conditions the rate of disappearance of reduced diphosphopyridine nucleotide (DPNH) is proportional to transaminase concentration. Five-tenths milliliter of 0.2M aspartate, 0.2 ml. DPNH (1 mg./ml.), 1.9 ml. 0.1M phosphate buffer at *p*H 7.4, and 5 μl. of malic dehydrogenase preparation (15 μg. protein) are mixed in a Beckman Model DU spectrophotometer cuvet. In the control cell DPNH is replaced by phosphate buffer. DPNH activity is measured by following the optical density at 340 mμ. When 0.2 ml. of serum is added, there is a rapid, and then a slower disappearance of DPNH, which is usually complete in 10 minutes but may take longer. At 30 minutes 0.2 ml. 0.1M α-ketoglutarate is added and the optical density is followed at 1- to 2-minute intervals. The linear portion of the curve corrected for the enzyme blank is used to calculate transaminase activity, 1 unit of which is defined as that amount of transaminase causing the optical density at 340 mμ to change at the rate of 0.001 per minute per cm. of light path. Replicate analyses agreed within 5 per cent. A modified Bausch and Lomb Spectronic 20 colorimeter can also be used. If a light path other than 1 cm. is used, the results should be corrected to conform to the units of Karmen, *et al.*

In 20 normal adults the serum transaminase values ranged from 10 to 33 units per ml. The levels are quite constant from day to day in a given individual and are not altered significantly by meals. Serum can be stored as long as 3 weeks in the refrigerator without loss of activity. Hemolysis greatly increases the activity of serum. Of 24 patients with myocardial necrosis, 22 had levels above 40 units within 48 hours after the onset of pain, with peak levels within 36 hours and normal values after 4 to 6 days. The peak values varied from 54 to 308 units/ml. Three subjects with the transient ischemia of angina pectoris without myocardial necrosis had no elevation. Marked increases were observed in diffuse liver disease and slight rises occurred after abdominal surgery.—*Proc. Soc. Exp. Biol. Med.* **89,** 31 (1955). (M. M. K.)

I^{131} blood levels correlated with gastric emptying determined radiographically: I. Protein test meal. G. J. Baylin, A. P. Sanders, J. K. Isley, W. W. Shingleton, J. C. Hymans, D. H. Johnston, and J. M. Ruffin (*Duke University School of Medicine, Durham, N. C.*).

Thirty-six normal human subjects who had been treated for 3 days with Lugol's solution were used. After a 6-hour fast, they were given a test meal of 50 μc. of I^{131}-labeled human serum albumin, 0.5 Gm. of gelatin per Kg. of body weight, and 225 ml. of tepid water. In 17 instances 20 Gm. of barium sulfate were added. Venous blood was taken at intervals for 3 hours and assayed for its I^{131} content. The position of the test meals with barium sulfate was determined radiographically. Similar studies were carried out with dogs, in which emptying of the stomach could be prevented by tightening a catheter placed around the pylorus. A characteristic and reproducible curve of blood radioisotope levels was obtained in both humans and dogs, which was altered by delayed gastric emptying, fainting, or change of position during the test.—*Proc. Soc. Exp. Biol. Med.* **89,** 51 (1955). (M. M. K.)

I^{131} blood levels correlated with gastric emptying determined radiographically: II. Fat test meal. G. J. Baylin, A. P. Sanders, J. K. Isley, W. W. Shingleton, J. C. Hymans, D. H. Johnston, and J. M. Ruffin (*Duke University School of Medicine, Durham, N. C.*).

Thirty-three normal human subjects previously treated with Lugol's solution and fasted 6 hours were given a test meal consisting of 50 μc. of I^{131}-labeled glycerol trioleate and 0.5 ml. of peanut oil per Kg. of body weight emulsified in an equal amount of water with 2–3 ml. of Tween 80. For 25 subjects 20 Gm. of barium sulfate were added. Venous blood was drawn at intervals for 4 to 6 hours and assayed for radioactivity. Eleven healthy dogs were similarly studied. No barium remained in the intestinal tract after 48 hours and during this time virtually complete absorption of the labeled material had occurred. A characteristic curve of radioactivity of the blood was obtained, the slope of which was influenced by the rate of gastric emptying. A low or flat curve could result from defective fat absorption or delayed gastric emptying.—*Proc. Soc. Exp. Biol. Med.* **89,** 54 (1955). (M. M. K.)

Now! A Primary Standard with PERMANENT STABILITY

For Clinical Analysis of Blood and other Biological Tissue

BIOSTAN®

Specially Prepared and Standardized

Bovine Blood Serum Ultrafiltrate

Use BIOSTAN as a primary standard to check the accuracy of the technician and the analytical method employed. READY TO USE!

BIOSTAN is a new, specially prepared and standardized bovine blood serum ultrafiltrate. It is intended for use in the clinical laboratory as a primary standard to check the accuracy of the technician and the analytical method employed. More than ten of the more common constituents in the blood and other biological tissues have been standardized in an analytical program organized by A. Saifer, biochemist of the Jewish Chronic Diseases Hospital of Brooklyn, with the assistance of sixteen additional supporting laboratories. The details of that study may be found by consulting the original literature (1).

METHOD OF PREPARATION

Nearly 200 gallons of freshly drawn bovine whole blood was defibrinated and then centrifuged to free it of the formed elements contained therein. The serum was next protected from organisms by the addition of 0.02% and 0.18% by weight of propyl and methyl esters, respectively, of p-hydroxy benzoic acid.

Then the serum was passed through a production unit of the International Biochemical molecular filter (2). The filter removed quantitatively all of the serum proteins and cholesterol but passed all other constituents whose molecular weight was 2000 or less. The filtrate obtained was pooled, its pH adjusted to 7.3 with carbon dioxide gas and then the whole batch was sterilized by filtration. It was next vialed aseptically.

At all stages precautions were taken to insure that the composition of all the vials were identical.

STANDARDIZATION

For the analytical standardization program the first vial and every 97th vial thereafter were withdrawn from the lot and coded so that the referee analyst would not know its position in the production run. These samples were sent to the seventeen laboratories participating in the standardization. The analyses obtained were carefully scrutinized to see that no trend developed in the data. The standardized values are given in the table (supplied with each vial).

Determinations of amino acid nitrogen, non-protein nitrogen, citric acid, magnesium, individual amino acids, etc., were reported. Too few of these were obtained to justify reporting a standardized value at this time.

INSTRUCTIONS FOR USE

1. Warm to room temperature immediately prior to use.
   Store vial in a refrigerator when not in use. If not contaminated Biostan will keep a long time under proper refrigeration.
2. Withdraw all aliquots with a dry and sterile syringe and needle. Do not return any excess to vial.
3. Except for calcium, sugar and uric acid where double quantities should be taken, the BIOSTAN should be treated exactly as if it were a blood or serum sample.

Because of the method used in preparing BIOSTAN, all of the low molecular weight constituents found in blood serum will be present and for the most part with a concentration identical to that in the blood. Since the lot manufactured is a large one the clinical chemist can use it as a reference standard for new analytical procedures with confidence that successive vials of this lot will be identical. Thus even if a constituent concentration is not known, as new methods of analysis are developed, the individual laboratory can standardize BIOSTAN for that particular constituent.

REFERENCE

(1) Saifer, A., and Deutscher, C. "A Study of Bovine Serum Ultrafiltrate as a General Standard in Clinical Analysis". Clinical Chem., January, 1956.

(2) Hiskey, C.F., and Kivert, A.N. "Apparatus for Molecular Filtration." Anal. Chem. Vol. 28 (1956).

ORDER BIOSTAN TODAY! 40-CC VIAL . . . . $10 each

STANDARD SCIENTIFIC SUPPLY CORP.

34 West 4th Street • New York 12, N. Y.

LABORATORY APPARATUS — REAGENTS AND CHEMICALS